A STUDY OF CK 19 EXPRESSION IN PAPILLARY LESIONS OF THYROID

Dissertation submitted in partial fulfilment of the

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PATHOLOGY

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TIRUNELVELI MEDICAL COLLEGE HOSPITAL

THE TAMIL NADU DR.M.G.R. MEDICAL UNIVERSITY,

CHENNAI,

TAMIL NADU

CERTIFICATE

This is to certify that the dissertation titled **"A STUDY OF CK 19 EXPRESSION IN PAPILLARY LESIONS OF THYROID",** is a bonafide work done by **Dr.T.GEETHA** Post Graduate Student, Department of Pathology, Tirunelveli Medical College, Tirunelveli – 627011, in partial fulfilment of the university rules and regulations for the award of MD DEGREE in PATHOLOGY BRANCH-III, under my guidance and supervision, during the academic period from 2015 to 2018.

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I hereby certify that this dissertation entitled "**A STUDY OF CK 19 EXPRESSION IN PAPILLARY LESIONS OF THYROID"** is a record of work done by **Dr.T. GEETHA**, in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during his postgraduate degree course period from 2015- 2018. This work has not formed the basis for previous award of any degree.

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DECLARATION

I solemnly declare that the dissertation titled **"A STUDY OF CK 19 EXPRESSION IN PAPILLARY LESIONS OF THYROID"** was done by me at Tirunelveli Medical College, Tirunelveli– 627011, during the period 2015 to 2018 under the guidance and supervision of **Prof.K.SHANTARAMAN, MD**, to be submitted to The Tamil Nadu Dr. M.G.R. Medical University towards the partial fulfilment of requirements for the award of MD DEGREE in PATHOLOGY BRANCH-III.

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CONTENTS

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PROTOCOL TITLE: A STUDY OF CK19 EXPRESSION IN PAPILLARY LESIONS OF THYROID.
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Dear, Dr.T.GEETHA, MBBS., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 10.12.2015.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
7.	Curriculum Vitae of the Principal Investigator	☐
8.	Insurance /Compensation Policy	☐
9.	Investigator's Agreement with Sponsor	☐
10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
12.	Clinical Trial Agreement (CTA)	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
14.	Clinical Trials Registry-India (CTRI) Registration	☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
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4. An annual status report should be submitted.
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CERTIFICATE - II

This is certify that this dissertation work title **"A STUDY OF CK 19 EXPRESSION IN PAPILLARY LESIONS OF THYROID"** of the candidate **Dr.T. GEETHA** with registration Number **201513303** for the award of M.D. in the branch of PATHOLOGY . I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion page and result shows **11 percentage** of plagiarism in the dissertation.

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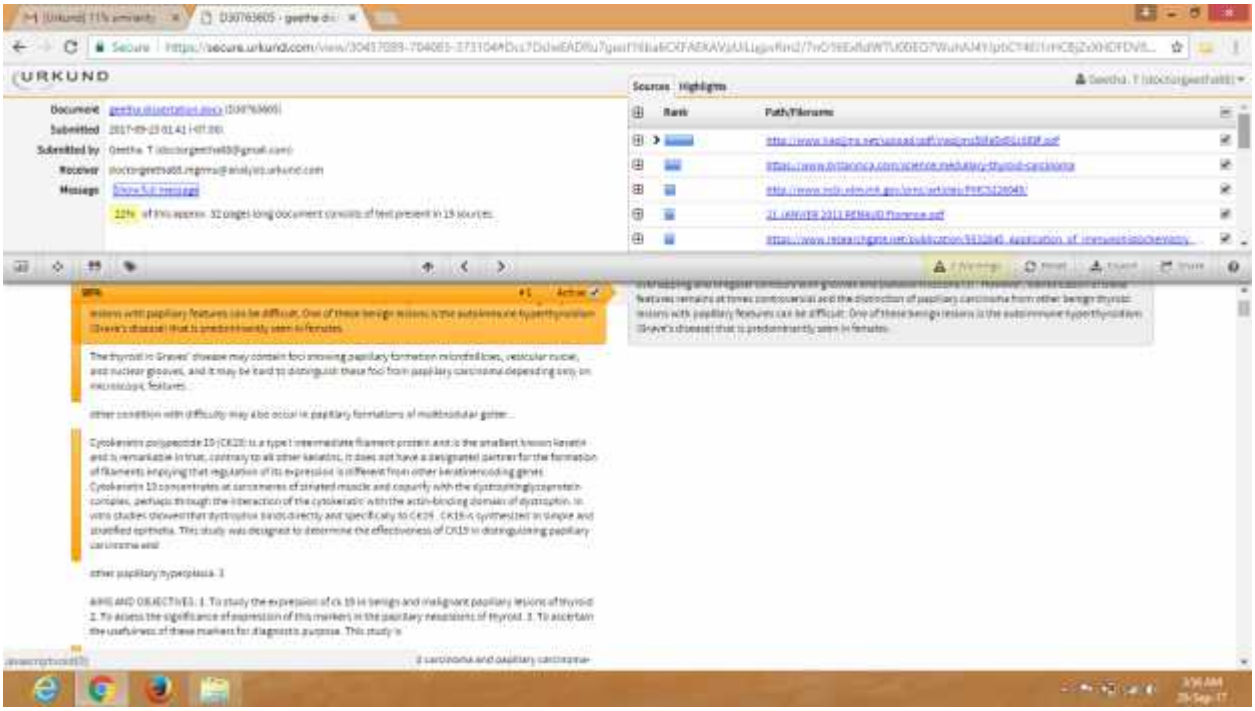
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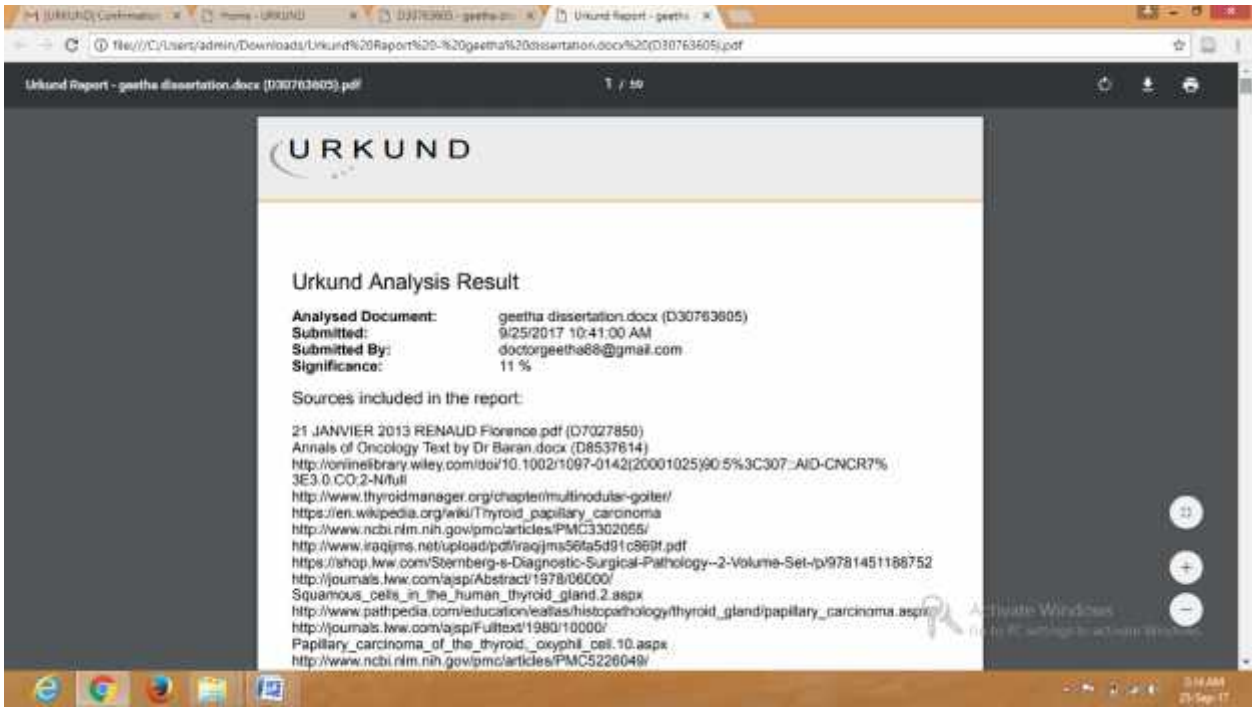
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INTRODUCTION:

Thyroid malignancies are the most common endocrine malignancy. The most common malignancy in thyroid are Papillary thyroid carcinoma. About 80% of all thyroid cancers are papillary carcinoma thyroid[1]**.** Papillary carcinoma typically arises as an irregular, solid or cystic mass that comes from otherwise normal thyroid tissue. Papillary carinoma has a high cure rate with 10-year survival rates for all patients with papillary carcinoma thyroid estimated at 80% to 90%.

The presence of lymph node metastasis in these cervical areas causes a higher recurrence rate but not associated with a higher mortality rate. Distant metastasis is rare, but lung and bone are the most common sites if the papillary carcinoma does spread. Tumors that invade or extend beyond the thyroid capsule have a much worse prognosis because of a high local recurrence rate.

Peak onset ages are 30 to 50 years old.Papillary thyroid carcinoma is most common in females than in males in a 3:1 ratio.The prognosis directly related to tumor size. That is tumor size is Less than 1.5 cm has a good prognosis.[2]

The identification of papillary thyroid carcinoma mainly on the presence of papillary architecture. The current accepted diagnosis of this entity is based on nuclear features that include optical clearing, elongation, overlapping and irregular contours with grooves and pseudoinclusions . However, identification of these features remains at times controversial and the distinction of papillary carcinoma from other benign thyroid lesions with papillary features can be

difficult. One of these benign lesions is the autoimmune hyperthyroidism (Grave's disease) that is predominantly seen in females. The thyroid in Graves' disease may contain foci showing papillary formation microfollicles, vesicular nuclei, and nuclear grooves, and it may be hard to distinguish these foci from papillary carcinoma depending only on microscopic features . other condition with difficulty may also occur in papillary formations of multinodular goiter .

Cytokeratin polypeptide 19 (CK19) is a type I intermediate filament protein and is the smallest known keratin and is remarkable in that, contrary to all other keratins, it does not have a designated partner for the formation of filaments implying that regulation of its expression, so it is different from other keratin encoding genes . Cytokeratin 19 concentrates at sarcomeres of striated muscle and copurify with the dystrophin glycoprotein complex, perhaps through the interaction of the cytokeratin with the actin-binding domain of dystrophin. In vitro studies showed that dystrophin binds directly and specifically to CK19 . CK19 is synthesized in simple and stratified epithelia. This study was designed to determine the effectiveness of CK19 to differentiate in distinguishing papillary carcinoma from other papillary hyperplasia.[3]

AIMS AND OBJECTIVES

1. To study the expression of ck 19 in benign and malignant papillary lesions of thyroid
2. To assess the significance of expression of this markers in the papillary neoplasms of thyroid.
3. To ascertain the usefulness of these markers for diagnostic purpose. This study is to determine the effectiveness of CK19 in distinguishing papillary thyroid carcinoma and papillary carcinoma-like changes other benign conditions like multi nodular goiter, graves disease and other lesions with papillary areas.

REVIEW OF LITERATURE

I. EMBRYOLOGY

Thyroid begins to develop between 2 to 3 weeks of gestation and completed by 11 week.[4]

-The gland develop from 3 structures

One median analage- develop from base of tongue to its final position in the anterior neck along the thyroglossal duct. Following the median analage descent and expansion to its final position, the thyroglossal duct then atrophies although the duct may persist, becomecystic in nature,&possibily even develop papillary thyroid carcinoma in thyroid tissue present in its wall. The two lateral analage develop from fourth to fifth branchial pouch, which contain utimobranchial body. Ultimobranchial body is associated with calcitonin secreting cells. Fusion of median and both lateral analage occurs in upper lateral aspect of gland.

II. ANATOMY :

The normal adult thyroid consists of two lobes connected by an isthumus. The thyroid gland usually located below and anterior to the larynx. The normal gland ranges from 14-18 g, depends on sex, size, and nutritional status of individuals. [5,6]The superior and inferor arteries supply the gland. Intraglandular and subcapsular lymphatics drain into the internal jugular lymph nodes. [5,7] Thyroid tissue is light brown in colour, firm in consistency.

III. Physiology

In response to hypothalamic factors , TSH is released by thyrotrophs in the anterior pitutitary into the circulation.The binding of thyrotropin to its receptor on the thyroid follicular epithelium results in activation of the receptor, allowing it to associate with Gs protein.Activation of G protein stimulates downstream events that result in an increase in intrcellular c AMP levels, which stimulates thyroid growth and thyroid hormone synthesis and release via c AMP- dependent protein kinases.

Thyroid follicular epithelial cells convert thyroglobulin into thyroxine and lesser amounts of triiodothyronin. [8]T4 and T3 are released into the systemic circulation, where most of these peptides are reversibly bound to circulating plasma proteins, such as thyroxin binding globulin and transthyretin.

The binding proteins act as a buffer that maintains the serum unbound T3 and T4 concentrations within narrow limits, while ensuring that the hormones are readily available to the tissues. The function of the thyroid gland can be inhibited by a variety of chemical agents, collectively reffered to as goitrgens. Because they suppress T3 and T4 synthesis, the level of TSH increase, and subsequent hyperplastic enlargement of the gland.

IV. HISTOLOGY

The thyroid tissue composed of follicles lined by epithelial cell that surround central colloid. 20 -40 follicles make up a lobule. Ultrstructral studies of normal thyroid shows that the follicular cells arranged in single layer around the colloid. [9]The cells contain liposomes and acomplement of endoplasmic reticulum,and small mitochondria.

The nuclei are round with homogenous chromatin. In the intersutium numerous fenestrated capillaries are noted. The c cells are found with in the confines of the basement membranes of follicles.

V. WHO (2017)

丿Tumours of the thyroid gland

- Follicular adenoma
- Hyalinizing trabecular tumour
- Other encapsulated follicular patterned thyroid tumours
- Tumours of uncertain malignant potential
- Noninvasive follicular thyroid neoplasm with papillary-like nuclear features
- Papillary thyroid carcinoma
- Follicular thyroid carcinoma
- Hürthle (oncocytic) cell tumours
- Poorly differentiated thyroid carcinoma
- Anaplastic thyroid carcinoma
- Squamous cell carcinoma

- Medullary thyroid carcinoma
- Mixed medullary and follicular thyroid carcinoma
- Mucoepidermoid carcinoma
- Sclerosing mucoepidermoid carcinoma with eosinophilia
- Mucinous carcinoma
- Ectopic thymoma
- Spindle epithelial tumour with thymus-like differentiation
- Intrathyroid thymic carcinoma
- Paraganglioma and mesenchymal / stromal tumours
  - Paraganglioma
  - Peripheral nerve sheath tumours
  - Benign vascular tumours
  - Angiosarcoma
  - Smooth muscle tumours
  - Solitary fibrous tumour
- Hematolymphoid tumours
  - Langerhans cell histiocytosis
  - Rosai-Dorfman disease
  - Follicular dendritic cell sarcoma
  - Primary thyroid lymphoma

Germ cell tumours

VI. PAPILLARY LESIONS OF THYROID:

Papillary thyroid lesions include papillary carcinoma, papillary hyperplasia, & papillary change in adenoma or adenomatous follicular nodule, medullary carcinoma with papillary areas.

a. PAPILLARY HYPERPLASIA:

It has been classified by their mechanism of production, morphologic feature and clinical manifestations.

a (1). Dyshormogenic goiter:

Goiter resulting from defects in hormone synthesis due to lack of responsiveness to TSH, defects in iodide transport ,ossification ,coupling , abnormalities in thyroglobulin synthesis. [10,11]

Grossly the gland is enlarged and multinodular.

Microscopical findings shows ,hypercellular nodules a variety of architectural apperances with a predominance of solid and microfollicular patterns. In some cases shows papillary and insular formation. Fibrosis with nuclear atypia and minimal amounts of colloid. [10]

Cases of thyroid carcinoma have beeen reported in patients with dyshormogenic goitre, but the number of well documented cases is very low. Most have been follicular type, others micropapillary carcinoma. [10]

Differential diagnosis:

Papillary carcinoma – differentiated by typical nuclear features.

Medullary carcinoma[11]

a. (ii) .Graves disease:

It is also known as basedow disease,thyrotoxicosis, diffuse toxic goiter. Commonly presents in young adult females, with muscle weakness , weight loss, irritability, tachycardia. Increased t4 and free t4 and bound t3 level increased.[12]

Grossly the gland shows a mild to moderate symmetric diffuse enlargement , reddish ,has a consistency of pancreatic tissue.

Microscopical finding shows the follicles are hyperplastic with prominent papillary infolding , it may confuse with papillary carcinoma. The lesion lined by columnar with basally located hypercromatic nuclei , and a clear cytoplasm, that may contain fat and glycogen. The colloid is pale, finely vacuolated with prominent scalloping. The stroma contains lymphoid aggregate.[13]Incidental carcinomas have been found in the glands removed for hyperthyroidism , incidence is 1 to 9 %.[14]

Graves disease is autoimmune disease, thought to be intiated by IgG antibodies against domain of TSH receptor.

Electron microscopy shows deposits of immune complexes in the follicular basement membrane.

a. (iii). Nodular hyperplasia:

It is an endemic goiter, due to low iodine content of the water and soil. Iodine deficiency leads to increased TSH secretion , which results in a hyperactive thyroid with tall follicular epithelium, and small amounts of colloid. Most commonly occur in adult population, incidence is 3 to 5%. [15]

Grossly , capsule may be stretched , multiple nodules with secondary change haemarrhage, calcification and cystic degeneration are common.

Microscopical finding shows varying appearances, some with large follicles lined by flattened epithelium, others have papillary projection, it may confuse with papillary carcinoma. Rupture of follicle may lead to a granulomatous reaction, prominent vascularisation. Thyroid nodules are clinically important for several reasons. They may cause thyroid dysfunction and, rarely, compressive symptoms, but they are primarily important because of the need to exclude thyroid cancer. The reported prevalence of malignancy in thyroid nodules evaluated by biopsy ranges from 4.0% to 6.5% and is largely independent of the nodule size. Despite this, papillary microcarcinomas (smaller than 1 cm) incidentally found at the time of surgery are much more common (up to 36%)[16,17]

Differential diagnosis:

- Papillary thyroid carcinoma - esp. papillary thyroid carcinoma follicular variant.
- Follicular thyroid adenoma - contained in a fibrous capsule.
- Follicular thyroid carcinoma - has fibrous capsule and invasion through it.[17]

b. PAPILLARY CARCINOMA THYROID

Thyroid carcinoma is the most common endocrine malignancy. It comprises 1% of all cancers. [18]

Thyroid cancer is divided into several main types, with papillary thyroid cancer being the most common. The treatment options for patients with thyroid

cancer include the surgical removal of the entire thyroid gland (total thyroidectomy), radioactive iodine therapy, and molecular-targeted therapies with tyrosine kinase inhibitors.[19]

This is the most common malignant tumor of the gland having iodine sufficient or iodine excess diet and comprises approximately 80% thyroid malignancies.(7) The tumors invade lymphatics leading to multifocal lesion. Most tumors are diagnosed in patients in the third and fifth decades. [20]Women are affected more than men in ratios of 2:1 to 4:1.[21]

b.(i).Etiologic factors: Etiologic factors for papillary carcinoma not well established. Various cellular and genetic mechanism and targets have been studied in the development of papillary carcinoma.

Iodine: The addition of iodine to the diet in endemic goiter areas in Europe and south America has been associated with a decreased incidence of follicular cancer and an increased in papillary carcinoma. Thyroid follicular cells proliferate only slowly under normal conditions, but in iodine deficient animals, serum TSH increases and the proliferation rate of thyroid cells increases by 5 to 30-fold, leading to marked thyroid hyperplasia and hypertrophy. Rapidly proliferating thyrocytes are likely more vulnerable to mutagens such as radiation, chemical carcinogens and oxidative stress, and may accumulate a higher number of genetic alterations. Thyroid hyperplasia induced by iodine deficiency results in chromosomal changes in the rat thyroid, with an increased number of aneuploid cells. Several authors have suggested that thyroid tumors caused by

iodine deficiency are due to chronic TSH overstimulation, possibly working together with epidermal growth factor and insulin-like growth factor I [22]

External radiation: External radiation probably plays an role in the development of papillary carcinoma. Papillary thyroid cancer with a relative risk incidence of approximately 80 % per se is typical for thyroid cancer in childhood and adolescence however, after exposure to radioiodine this relative frequency is increased close to 100%. Latency times between radiation exposure and development of thyroid cancer, range between a minimum of 3-7 years and a maximum of 40-50 years. Risk decreases significantly with increasing age of exposure with little risk apparent after the age of 20 years.[23]

The great increase in the incidence of papillary carcinoma in Belarus and Ukraine has been apparent since the Chernobyl nuclear accident.[23] Most reported tumors following this accident have been papillary carcinoma. Many of which show aggressive histologic features including extracapsular invasion and vascular invasion.

Auto immune disease : Patients with graves disease have a higher than expected incidence of papillary carcinoma. [13]Many studies indicate that upto one third of papillary carcinoma arise in the setting of chronic thyroiditis. [24]However recent molecular data have shown that foci of atypical follicular epithelium in chronic thyroidits do have loss of heterozygosity for various tumor suppressor gene and RET /PTC rearrangements.[25]

Hormone and reproductive factors: Papillary carcinoma have been described in patients with familial adenomatous polyposis coli, cowden syndrome , hereditary nonpolyposis colon cancer syndrome, peutz- jeghers syndrome and ataxia tenangietasia. Familial adenomatosus polyposis coli is caused by germline mutation of the adenomatosus polyposis coli gene. Thyroid carcinoma mostly papillary carcinoma occurs in 1-2 % of patients with familial adenomatosus polyposis coli, all these show germ line mutation of adenomatosus polyposis coli gene.[26]

However somatic mutation or loss of heterozygosity for adenomatosus polyposis coli gene are not found in thyroid tumors. A majority of these tumors do show activation of RET/PTC1 in thyroid tumors, suggesting a possible association between APC and RET/PTC in the development of this particular subset of familial papillary carcinoma.[26]

Cowden syndrome is characterized by formation of hamartomas in several organ and a high risk of developing breast and thyroid cancer. Genetic loss for cowden syndrome has been mapped to chromosome 10q23.3 and is also known as PTEN .[27,28] PTEN mutation or gene deletion is noted in 26% of benign tumors , but only in 6% in malignant tumors of thyroid[28]. 28

Thyroid and parathyroid adenomas

Occasionally papillary carcinoma arise in benign nodules or adenomas . Papillary carcinoma and parathyroid adenoma or hyperplasia, both types of lesions are associated with a history of low dose external radiation to the neck.

Pathology of papillary carcinoma thyroid:

Gross appearance of papillary carcinoma is variable.

The lesion may appear anywhere in the gland. By definition, typical papillary carcinoma are greater than 1 to 1.5 cm often averaging to 2 to 3 cm, although lesions may be quite large.[2] The lesions are firm and usually white in colour with an invasive appearance. Lesional calcification is a common feature. Necrosis is a not a feature of typical papillary carcinoma and suggests a higher grade lesion. HT is the most prevalent autoimmune disease and one of the most common endocrine diseases. This condition is the most common cause of hypothyroidism, excluding cases secondary to thyroidectomy, that are predominant among females. The association between PTC and HT was first described in 1955 by Dailey et al., and became evident because of an increase in new cases of thyroiditis diagnosed by anatomopathological exams over the past decades. The concept of chronic inflammation as a risk factor for the development of malignancies has been well established for other tumors. However, with respect to these two entities, the association of cause and effect between them remains uncertain.[29]

Microscopical finding of shorts certains features of papillary carcinoma . The neoplastic papillae contain a central core of fibrovascular tissue lined by one or occasionally several layers of cells with crowded oval nuclei. In papillary hyperplasia thyroid follicles may some time exaggerate into papillary

change,there is infolding of the lining epithelium composed of columnar cells with basal round and uniform nuclei.

There is either no central core or a core of edematous or myxomatous paucicellular stroma often including small follicles. Papillary carcinoma, psammoma bodies that represent the ghosts of dead papillae are differentiated from dystrophic calcifications by lamellations. True psammoma bodies are formed by focal areas of infarction of the tips of papillae, attracting calcium that is deposited on the dying cells.[2] Psammoma bodies are usually present with in the cores of papillae or in the cores of papillaeor in the tumor stroma but not in the neoplastic follicles. The finding of psammoma bodies in a cervical lymphnode is astrong evidence of papillary carcinoma in the thyroid.

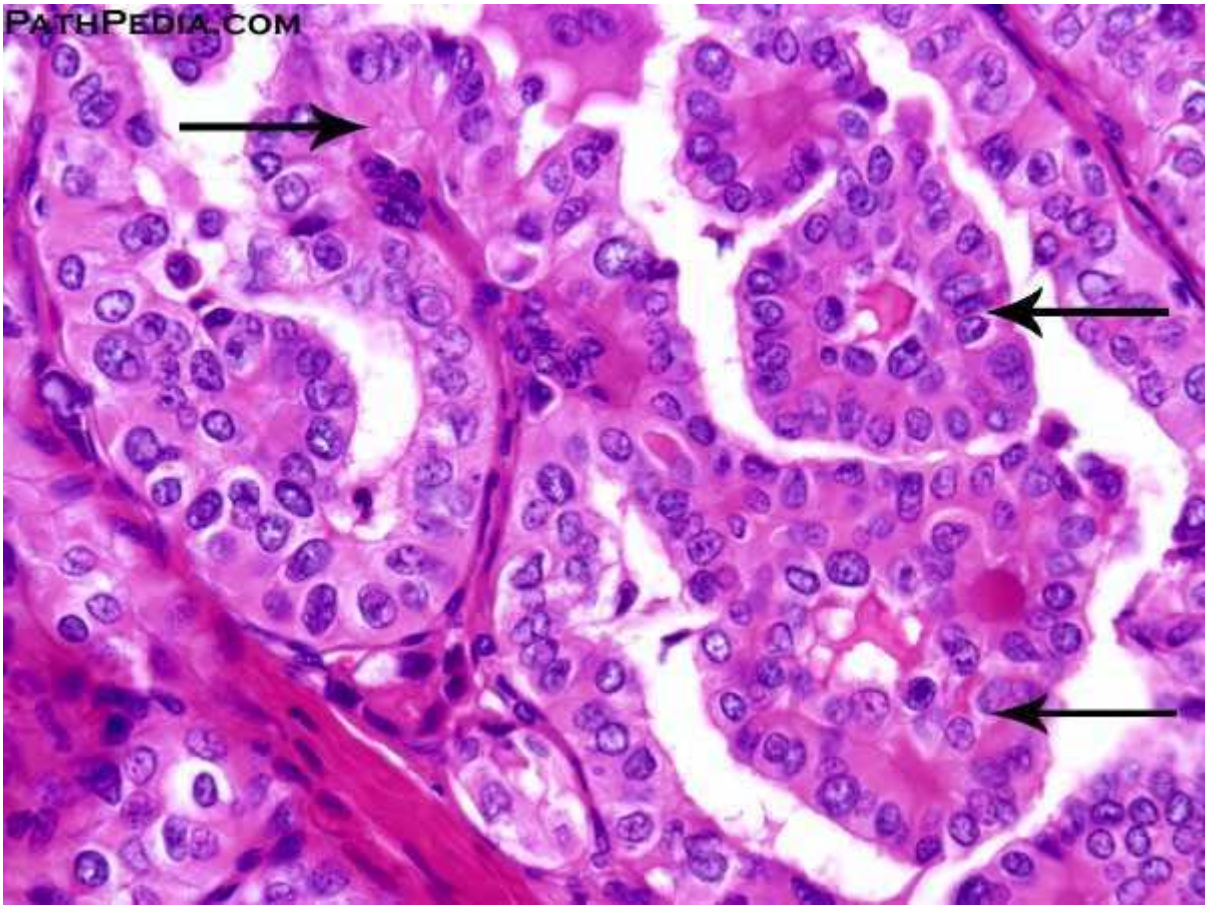
The nuclei of papillary carcinoma have been described as clear, ground glass,empty or orphan annie eyed nuclei. These nuclei are larger and moreoval than normal follicular nuclei, and contain hypodense chromatin.(21)

In papillary carcinoma these nuclei are often overlap one another. Although cleared nuclei are characteristic of papillary carcinoma, autoimmune thyroiditis, particularly often shows similar nuclear changes. Intranuclear inclusions of cytoplasm are often found. Another characteristic of the papillary carcinoma nucleus is nuclear groove. Nuclear grooves may be seen in other thyroid lesions including hashimotos disease, adenomatous hyperplasia and diffuse hyperplasia as well as follicular adenomas. For the most part nuclear

grooves more commonly seen in papillary carcinoma ,mere presence of nuclear grooves is not a diagnostic of papillary carcinoma.

Many of papillary carcinoma contain foci of squamous differentiation approximately 15 t0 45%.[30]. Almost all papillary carcinoma shows areas of desmoplasia either in the central portion of the tumor or at the peripheral zones of the lesion. Cyst formation may occur in fact may be so striking that the diagnosis of papillary carcinoma is difficult to make, particularly if the lesion has metastasized to neck lymph nodes making the distinction from a branchial cleft cyst difficult. Papillary carcinoma invades the glandular lymphatics, which accounts for high incidence of regional node metastases. [31,32]Regional lymph node metastases are extremely common at initial presentation of usual papillary cancer. This feature does not affect long term prognosis. Papillary carcinoma can also present as multifocal tumors with in the same gland . it has been shown that papillary carcinoma are clonal proliferation. [32]

Venous invasion can be identified in up to 7% of papillary cancer. It has been suggested that cases with histologic vascular invasion may be considered as a sign of an increased tendency toward haematogenic invasion and consequent increase in the relative percentage of metastases. [32]Distant metastases of papillary carcinoma to lungs and bones occur in less than 10 %. despite the presence of multiple metastases ,however survival may still be prolonged. Especially if the metastases can be treated with radio iodine. In ordinary papillary carcinoma death is uncommon. [33]



[34]figure 1. Papillary carcinoma thyroid histopathology

The electron microscopic appearance of papillary carcinoma includes a nucleus with dispersed chromatin and highly infolded nuclear membrane,cytoplasmic intrnuclear inclusions , and a cytoplasm that contain many mitochontria and numerous cytoplasmic filaments. [2,35]Keratohyaline granules have been found in tumors with squamous foci.

b.iii.VARIANTS OF PAPILLARY CARCINOMA

1.Follicular variant: follicular variant of PTC (FVPTC) is the most common subset of papillary carcinoma and is found in 9% to 22.5% of patients with PTC [36] This variant is composed entirely of follicles. These lesion show an infiltrative

growth pattern, or an encapsulated pattern. The follicles vary in size and shape are often elongated or irregular shaped with abortive papillary formation, and the colloid is usually deep stainedand scalloped.

Psammoma bodies and sclerosis may be present. The diagnosis made by identification of the typical nuclear features. Follicular variant of papillary carcinoma has two entities ;

1. Infiltrative type
2. Encapsulated type.

The infitrative non encapsulated type shows obvious infiltration of thyroid parenchyma often accompanied by sclerosis. The growth is similar to that of conventional papillary csarcinoma, except the papillae is absent. The encapsulated type is surrounded by a fibrous capsule ,and it may or may not exhibit invasion of the capsule or blood vessels.

Clinically low frequency of lymphnode metastasis; encapsulated solitary tumor , infrequent intratumoral scelerosis. Presence of RAS mutation or **PAX8**-PPARγ [37] translocation

Kakudo and colleagues , recently proposed the name well differentiated thyroid tumor of uncertain behaviour to encompass this variant and encapsulated follicuar patterend tumors with features of papillary carcinoma.

Solid variant

This variant shows more than 50% solid, or trabecular growth pattern. The tumor is traversed by delicat capillaries and the nuclear features of conventional papillary carcinoma.[38,39]This variant occurs with a disproportionate frequency among nuclear accident associated papillary thyroid carcinoma. It is associated with high frequency of distant metastasis and less favorable prognosis. [40]

Encapsulated variant

This variant constitutes 4% to 14% of all papillary carcinoma.[41,42] The fibrous capsule may or may not show invasion by tumor, but lymph node metastasis can occur even in the absence of capsular or vascular invasion. The patients tend to be younger,and the frequency of lymphnode metastasis is lower .[42]The prognosis is excellent.[43] The findings that the encapsulated follicular variant of papillary carcinoma behaves more like follicular adenoma and carcinoma.

2.Diffuse sclerosing variant

This rare variant mostly affects children[44] and young adults. Patients present with unilateral or bilateral symetrical thyroid swelling. Serum anti thyroglobulin or antimicrosomal antibodies may be positive . [45]This variant shows more aggressive than conventional type, as manifested by higher incidence of extra thyroidal extension ,lymphnode metastasis and distant metasis.[44,45]

The thyroid shows diffuse replacement of the parenchyma by white firm tisue,which isoften gritty on cutting.

The typical histologic features include

1. Diffuse involvement of one or both obes.
2. Scelerosis
3. Heavy lymphoplasmacytic infiltrate.
4. Abundant psammoma bodies.
5. Scatterd small islands of papllary carcinoma with prominent squamous or squamoid differentiation.
6. Extensive lymphatic permeation.[44]

3.Diffuse follicular variant

This is a rare aggressive form of papillary carcinoma, it occurs in young patints.[15,46] This variant is characterised by diffuse enlargement of the entire thyroid without formation of distinct nodules, predominent follicular pattern, and absence of fibrosis. Histology showed a well-differentiated thyroid cancer with a diffuse follicular growth pattern, mainly constituted by micro-follicles containing a small amount of colloid, organized in confluent nodules of small to medium size with dense collagenous septa without a detectable tumor capsule.[46] The diffuse follicular variant shows a high frequency of lymph node ,pulmanory and bone metastasis.[47] it is a clinically aggressive tumor and had a poor prognosis. [47]

4.Tall cell variant

This variant composed predominantly of cells with height at least three times their width compared with conventional papillary carcinoma proposed by WHO. [48] This type shows following features,

1. Slightly older age group (50-57 years)[48]
2. Bulkier tumors
3. Higher frequency of BRAF mutation.(80%) [48]
4. More likely to show extrathyroidal extention(42-82%)[49]

5.More aggressive [49]

One third of cases ehibit RET/PTC rearrangements, selectively RET/PTC3[50] exhibits more potent than RET/PTC 1. The tall cell variant is highly papilliferous and invasive. The nuclei are typical those of papillary carcinoma conventional type,are mostly basally located. The cytoplasm is plentyful and oxyphilic because of accumulation of mitochondria.Focal clearing of the cytoplasm is some times present.

5.Columnar cell variant

The columnar cell variant was first reported by evans as a rare thyroid neoplasm.[51] More aggressive than differentiated thyroid carcinoma. [52]The tumors are invasive. The Mean age is 57 years. [53] A more frequently metastases to lung vertebra, and regional lymph node metastases exists. [51]The mortality rate is high. This type is characterised by

1. Mixed papillary ,glandular,cribriform and solid patterns.
2. The papillae and glands are lined by tall columnar cells with pseudostratified hyperchromatic oval or elongated nuclei.
3. Sub nuclear vacuoles and cytoplasmic clearing may be present.
4. The cells in the solid areas are often smaller and polyglonal.
5. The tumor is thyroglobulin positive. [54]
6. BRAF Mutation is demonstrable in one third of cases.[53]

6.Oxyphilic variant:

The latest World Health Organization International Classification defines papillary thyroid carcinoma by its "follicular cell differentiation, as well as characteristic nuclear changes". However the oxyphilic (Hürthle cell) papillary carcinoma have nuclei which generally resemble the nuclei seen in oxyphilic follicular carcinomas, and such oxyphilic papillary tumors may behave more aggressively than typical papillary cancers[55]. The tumor is formed predominantly by cells with abundant eosinophilic granular cytoplasm by accumulation of mitochondria. It is important to differentiate this neoplasm from Hürthle cell follicular neoplasms of thyroid .[56]

7.Warthin tumor like variant

Rare variant of papillary carcinoma, features similar to warthin tumor of salivary gland.The tumor composed of a papillary pattern and a rich lymphoplasmacytic infiltrae in the cores of papillae. [57]The cells that cover the papillae are tall and oxyphilic having the abundant cytoplasm .

8.Clear cell variant :

Rare variant of of papillary carcinoma.[58] These tumors comprised of mainly clear cells have a papillary architecture and cytological features of PTC. Some tumors may have oncocytic and clear cell features . Clear cell appearance believed to be due to mitochondrial expansion or accumulation of glycogen or mucin Electron microscopy reveals dilated empty mitochondria.

Also believed to be related to TSH overstimulation[58]. Immunostaining for TTF-1 and thyroglobulin may be necessary to distinguish these tumors from a metastatic clear cell carcinoma.[57]

9.Macrofollicular variant

This type has the more than 50% of their area composed of large follicles. [59]The cells that line the macrofollicles are attenuated and may not show the characteristic nuclear features of papillary carcinoma. Assesement is made on the smaller follicles.Uncommonly, dedifferentiation to undifferentiated carcinoma can occur. [60]

10.Trabecular variant

This variant shows the trabecular growth pattern ,more than 50% of cells. The cells are cuboidal or columnar, with cells perpenticular in long and straight trabeculae. The tumors are large and invasive. This variant has been associated with poor prognosis. This variant has also been considered by some authors as a poorly differentiated variant of papillary carcinoma.differential diagnosis include, Hyalinizing trabecular tumors are rare tumors of the thyroid gland

typified by a trabecular or alveolar architecture with extensive hyaline and colloid deposits. Their cellular features of cytoplasmic inclusions, nuclear pseudoinclusions, nuclear grooves, and psammoma bodies often cause them to be mistaken for papillary thyroid carcinomas on FNA. They represent benign or low-malignant-potential tumors that are adequately treated by thyroid lobectomy.[61]

11.Cribriform morular variant

This is a uncommon variant. It is microscopically shows prominent cribriform pattern,with interspersed squamoid islands. The nuclei filled with lightly eosinophilic ,homogenous ,biotin containing inclusions. Cells arranged in closely packed follicles ,papillae, trabeculae. The characterstic feature ,the luminal spaces shows absence of colloid. The tumor cells are cells are columnar or cuboidal. The nuclei are chromatin rich, typical of nuclear features of papillary carcinoma can often seen focally.

Some of cells showing spindle shaped nuclei,forms the fascicles and whorls.

The tumor shows circumscribed, encapsulated,with or with out capsular or vascular invasion. This variant of papillary carcinoma can occur as a sporadic tumor , or associated with familial adenomatosus polyposis coli syndrome.

Female predominance (1:17), the mean age of diagnosis is 27.7 years. The outcome of this tumor is favorable. This type commonly show RET/PTC rearrangement.[62]

The APC gene shows either germline or somatic,germline mutation in FAP associate cases, somatic mutation in some sporadic cases. Somatic mutation in exon 3 of the beta catenin gene, which results in nuclear translocation of beta catenin. BRAF mutation is not found in this type. Cytokeratin 19 (CK19) is commonly strongly expressed in papillary thyroid carcinomas. It is also positive in the squamous metaplasia in diffuse sclerosing variant of papillary thyroid carcinoma . In contrast, CK19 may express only weakly in the morules in CMV-PTC. Cytoplasmic staining of CD10 and nuclear staining of bcl-2 are strongly positive in the morular cells but weak or not positive in the non-morular area .[63]

12.Papillary carcinoma with lipomatous stroma

In rare circumstances , adipose cells are interspersed with in the papillary carcinoma. [64]

13.Variant with exuberant nodular fascitis like stroma

It is a rarely ,papillary carcinoma associated, with an abundant nodular fascitis or fibromatosis like stroma. Microscopically shows the stroma composed of spindle cells lying in a vascularised fibromyxoid matrix with extravasated red cells. The spindle cells are myofibroblastic Ŧhese cells had oval to elongated nuclei with fine chromatin and small distinct nucleoli . There was no pleomorphism, and mitotic figures were not identified. Some extravasated red blood cells were present in addition to interspersed lymphocytes and mast cells. The interaction between the stroma and tumor results in unusal histologic pattern like fibroadenoma and phylloides tumor of breast. The carcinomatous component showed positive immunostaining for thyroglobulin and cytokeratin but not for

calcitonin. Cytokeratin staining highlighted occasional isolated tumor cells in the stroma. Some tumor cells also showed vimentin and S-100 protein positivity. The spindle cells were positive for vimentin and muscle-specific actin; a small proportion also stained for desmin. They were negative for S-100 protein.[65]

14.Variant with spindle cell metaplasia

Rare variant of papillary carcinoma shows a component of spindle tumor cells, constitute a major or minor proportion. The bland looking spindle cells form short fascicles, along with the papillary carcinoma component.

This case reported as spindle cell transformation of papillary carcinoma is a dedifferentiated papillary carcinoma rather than an example of this variant. [66]

15.Hobnail variant

It is a aggressive and rare variant of papillary carcinoma. Commonly patient present with Cervical lymphadenopathy . Distant metastasis are common. The tumor is multifocal, with microscopic finding shows variably sized papillae covered by cells with atypically placed nucleus ,forming a surface bulge. BRAF mutation commonly present in about half of the cases.[67]

16.Micropapillary variant

This is a rare type and having a prognosis. It is most commonly present with lympho vascular invasion, lymph node metastasis, and distant metastasis are seen. This variant is microscopically shows the , micropapillary growth pattern with out fibrovascular cores, more than 5% of the tumor area.[68]

17.Adenoid cystic carcinoma like variant

Papillary carcinoma rarely present with abundant deposits of globular hyaline material in focal areas of tumor, like a adenoid cystic carcinoma of salivary gland. Fine-needle aspiration smears from the thyroid nodule were highly cellular and showed follicular cells arranged inpapillary clusters and in monolayered sheets with d cytoplasmic vacuolization, including marginal vacuoles. In some areas, structures resembling follicles with central hyaline globules, reminiscent of adenoid cystic carcinoma increased frequency of nuclear grooves. In May-Grun wald-Giemsa-stained smears, the neoplastic cells were cuboidal to low columnar with clear to light-red cytoplasm an. These globules were pink to magenta in colour.(68)

18.Dedfferentiated papillary carcinoma

This type coexistenceof papillary carcinoma with an undifferentiated or poorly differentiated thyroid carcinoma. This transformation can occur either in primary tumor or metastatic deposits. The prognosis is bad.

19. Microcarcinoma

It is also called as papillary microtumor.

It is defined as a tumor less than 1 cm.It has a excellent prognosis even in lymphnode metastasis or distant metastasis cases. Rare cases with microcarcinoma who have an unfavorable outcome are those with lymphadenopathy greater than 3 cm , and a nonencapsulated type of papillary lesion. [69]

In the 2004 WHO classification ,the definition of micropapillary carcinoma, include only a incidental finding with less than 1 cm but not clinically evident small papillary carcinoma.

b.iii.MOLECULAR PATHOLOGY OF PAPILLARY CARCINOMA: There are three molecular alterations are recognised in papillary carcinomas, and result in activation of the mitogen activated protein kinase path way.

1. RET mutation
2. BRAF Mutation
3. RAS Mutation

Activation of the proto oncogene ret or trk by intrachromosomal inversion or chromosomal translocation

This occurs in 10 to 30 % of cases. Transfection of primary cultures of human thyroid epithelial cells with a RET/PTC retroviarl construct results in nuclear changes including irregular nuclear contour and euchromatic appearance, suggesting that the genetic alteration may cause the characterstic nuclear feature of papillary carcinoma. Oligoglonal RET/PTC rearrangement can occur in non neoplastic thyroid tissues such as hashimotos thyroditis and benign nodules.

The tyrosine kinase of RET gene can be fused with a number of genes constitutively expressed in thyroid epithelial cells, such as PTC -1, THROUGH INV (10) **(**q11.2q21), PTC 2 through t(10;17),q(11.2q23),PTC 3through cytogenetically undetectable paracentric inversion within 10q11.2,PTC4,PTC 5. The fusion contributes a dimerization domain to the RET protein, leading to

activation of the tyrosine kinase.RET/PTC 1 is most common,followed by RET/PTC 3.[70]

The frequency of RET/PTC gene fusion is higher among children and young patients,(50- 60%), chernobyl accident associated papillary carcinoma(60 -80%) , mostly RET/ PTC 3, and who had received external radiation therapy (60- 80%),[70] most commonly RET/PTC 1. RET/PTC 1 is correlated with papillary carcinoma with predominate papilary structure and papillary microcarcinoma.RET/ PTC 3 is correlated with the tall cell and solid variants.

BRAF mutation

BRAF belongs to RAF family of protein kinase that play a role in transduction of signals along the RAS/RAF/MEK/MAPK pathway,mediating cell growth, differentiation and survival. Some study from, Animal experiments discovered that normal rat thyroid cells with *BRAF* V600E mutation were likely to promote tumor invasion by expressing certain gene products and degradating the extracellular matrix of PTC.[71,72]

In addition, researchers also found that the *BRAF*V600E mutation in PTC was correlated with transportation and metabolism of iodide, and resulted in radioiodine treatment failure . Furthermore, the *BRAF* V600E mutation induced silence of several tumor-suppressor genes and further promoted the invasiveness of PTC. Xing et al discovered that the *BRAF* V600E mutation in PTC was correlated with methylation of following tumor suppressor genes including tissue inhibitor of metalloproteinase-3 (TIMP3), SLC5A8, death-associated protein kinase (DAPK), and retinoic acid receptor b2 (RARb2).

The most common mutation is a missense mutation at nucleotide 1799 with T-A transversion that results in substitution of valine by glutamic acid. The frequency of BRAF V600E mutation in papillary carcinoma has risen gradually over past two decades.

BRAF V600E mutation is less in less frequent in papillary carcinoma of children and young patients.[73]

The prognontic significance of BRAF mutation is controversial, but most studies report this features to be correlated with aggressive features such as old age ,extrathyroidal extension , more advansed stage,, lymph node metastasis, and tumor recurrence.

RAS mutation

Mutation of the RAS gene is reported approximately 15% of papillary carcinoma , and the all positive cases were follicular variant. Mutations in *RAS* is the the second most commonly identified genetic alteration in thyroid malignancies. *RAS* mutations are primarily found in follicular-patterned tumors, including Follicular adenoma, follicular cancer, and the follicular variant of papillary cancer. There is increasing evidence that *RAS* mutation status has significant diagnostic utility when used concurrently with FNAB.[74]

A small proportion of cases of the follicular variant of papillary carcinoma (7%) show a mutation in the BRAF gene different from V600E , with substitution of lysine by glutamate at codon 601. (k 601E).

b.iv. PROGNOSTIC FACTORS

1. Age : Papillary carcinoma mostly developing in children and adolescents having good prognosis. Nearly all deaths from papillary carcinoma commonly occur when it is occur after the age of 45 years[75].

2. Sex: Better prognosis in females than males.

3.Extra thyroidal extension : papillary carcinoma with extrathyroidal extension having the bad prognosis.

4.Previous irradiation : it does not seem to significantly differ from others.

5.Tumor size: inverse correlation is present between size and prognosis.

6.capsule and margins : Better prognosis in encapsulated tumor.

7.**Multicentricity and distant metastasis :** patients with metastasis having the bad prognosis;

8.Poorly differentiated ,squamous or anaplastic foci: bad prognosis. It has only 5% of presentation.

9.DNA ploidy : it has a good correlation between a aneupliody and having an aggressive behaviour in papillary carcinoma.

10.BRAF: BRAF mutation shows aggressive tumor. Unresponsive to radioactive iodine. [76]

c.MEDULLARY CARCINOMA :

Medullary carcinoma is a rare , 10 % all thyroid malignancies. The tumor is a aggressive in nature. It most commonly associated with MEN 2A, MEN 2B, familial non- MEN MTC. C cell hyperplasia is a precursor lesion. Most of medullary carcinoma occur sporadically, 10 to 20 % are familial . It is

associated with mutations in RET oncogen, identified on chromosome 10. It occur at any age group ,mostly adults in the age group of 50 years. children may be affected in familial medullary carcinoma. It commonly Present with painless firm nodule. Nodal metastasis are common. Distant metasasis to lung , bone are liver.

Pathology: Medullary carcinoma arises in the area of more on c- cell concentration, lateral upper 2/3 of gland. In familial cases present with multiple small nodules. Grossly it is a circumscribed, focal areas shows necrosis and haemorrhage. **Medullary thyroid carcinoma,** tumor of the para follicular cells (C cells) of the thyroid gland. It occurs both sporadically and genetically, affecting multiple members of families who carry gene mutations associated with the disease. In some families medullary thyroid carcinomas are the only tumor that appear, whereas in other families medullary thyroid carcinomas are associated with multiple endocrine neoplasia type 2 (MEN2). Medullary thyroid carcinomas are moderately malignant tumors that invade nearby tissues in the neck and spread to distant organs, such as the lungs and liver. A characteristic feature of these tumors is hypercalcitoninemia, an abnormally high serum concentration of a protein hormone called calcitonin, which is secreted by C cells. Calcitonin normally lower the concentration of calcium in the blood when it rises above the normal value. However, despite marked increases in serum calcitonin concentrations, patients with medullary thyroid carcinoma do not have low serum calcium concentrations (hypocalcemia), because their tissues are resistant to calcitonin. Nearly all patients affected by medullary thyroid

carcinoma or MEN2 have hereditary mutations in the *RET* (*re*arranged during *t*ransfection) proto-oncogene (a gene that can become a cancer-causing gene, or oncogene). Patients with medullary thyroid carcinoma should be analysed for mutations in *RET*; if a mutation is detected, other family members should also be tested. Some people with hereditary mutations in *RET* will develop medullary thyroid carcinoma at a young age. Therefore, any individual carrying a *RET* mutation usually undergoes thyroidectomy (removal of the thyroid gland) at an early age, before a tumour appears.[77]

Microscopically , circumscribed, cells arranged in nest pattern, separated by stroma. Cells being round ,oval or spindle shaped cells. Nuclei are uniform. Nuclear cytoplasmic ratio is low. Intranuclear inclusion, mitotic figures will be present.The stroma contains amyloid. Variants have been papillary variant, papillary or pseudopapillary growth is paresent. It should be differentiated from tyoical papillary thyroid carcinoma by typical nuclear features.

VII. DIFFERENTIAL DIAGNOSIS OF PAPILLARY LESIONS OF THYROID.

Fine needle aspiration cytology:

Fine needle aspiration cytology of the thyroid has been increasingly utilized for the investigations of thyroid lesions. Since cancer is more common in solitary cold nodules, prevalence of malignancy in solitary cold nodules ranges from 10% to 44.5 % [78]

The main indications of fine needle aspiration in thyroid lesions are the following[79]

1. Evaluation of solitary thyroid nodules with a view to distinguish benign from malignant.
2. Evaluation of diffuse thyroid lesions with a view to distinguish inflammatory and autoimmune lesions from nodular goiter
3. Confirmation and categorization of clinically obvious thyroid malignancy. especially anaplastic carcinoma that may require preoperative palliative treatment, and lymphoma and metastatic malignancy where surgery is usually not indicated.
4. To obtain material for ancillary test and prognostic parameters.
5. Evaluation of lesions detected initially by imaging, measuring 1-1.5 cm diameter with features suspicious of malignancy.

Fine needle aspiration has been shown to the safest and most accurate of diagnostic tools in thyroid lesion. Reporting of thyroid fine needle aspiration specimen should follow a standard format that is clinically relevant in order to direct management. At the national cancer center institute sponsored thyroid state of the science conference in Bethesda in October 2007. Consensus was reached regarding indications, pre-fine needle aspiration, techniques, diagnostic terminology. The Bethesda system reporting terminology includes six categories

1. Non diagnostic
2. Benign
3. Atypia of undetermined origin
4. Suspicious of follicular neoplasm
5. Suspicious for malignancy

6. Malignant.

Cytologic diagnosis is generally accurate in thyroiditis, usual type of papillary carcinoma, medullary carcinoma, anaplastic carcinoma and high grade lymphoma. False negatives have been minimized by using ultrasound guided fine needle aspiration.

Normal structure: Follicular epithelial cells and colloid are regular features in normal thyroid and in colloid goiter. thyroid and in colloid goiter. Follicular cells show fragile blue cytoplasm or pale blue cytoplasm with indistinct or fuzzy cell border. Coarse blue cytoplasmic granules may be seen. Thick colloid appears as round, dense clumps, of deep blue, violet, or magenta coloured acellular materal.

FINE NEEDLE ASPIRATION CYTOLOGY

LESIONS	FNAC FINDINGS	DIFFFERENTIAL DIAGNOSIS
Nodular goiter	Flat monolayered sheets of epithelial cells has frayed edges. Abundant thick and thin collid. Cytoplasm is indistinct. [80]	Cystic papillary carcinoma- May contain abundant colloid. can cause diagnostic difficulties. Hyperplastic papillae containing follicles and intact dilated follicles in cell block preparation shows a beningnity of nodule.
Graves disease	Colloid free bloody background.monolayered sheets with moderate amount of pale cob web like vacuolated cytoplasm. [81]	Papillary carcinoma- colloid free, with nuclear atypia mimic papillary carcinoma. It can be differentiated by papillary carcinoma shows denser cytoplasm

		with well defined margins .
Papillary carcinoma	Flat sheets ,three dimentional structures, and papillary fragments ,shows anatomical bordering. Enlarged ovoid nuclei ,fine granular powdery chromatin in PAP stain. Chewing gum colloid. Intranuclear cytoplasmic inclusions and nuclear grooves . psammoma bodies variable. [82] Positive immunostaining for CK 19, CD 44, HBME 1.	Papillry foci are present in hyperplastic nodular goiter and graves didease.
Cysti papillary carcinoma.	Large cell size, pseudo inclusions, nuclear grooves, well defined vaculoes in atypical histiocytoid cells. [79]	Histiocytic cells in cystic nodular goiter mimic papillary carcinoma. Immunostainig for CD 68 may be useful.
Follicular variant of papillary carcinoma	Follicular architexure, nuclear features shows powdery pale chromatin, and nuclear grooves.	Follicular neoplasm-combined use of hbme 1 and ck 19 may be useful to distinguish between follicular neoplasm and follicular variant of papillary carcinoma.
Macrofollicular encapsulated variant	Predominant macrofollicles, large, cuboidal cells with fine nuclear chromatin, grooves, pseudo-inclusions and dense eosinophilic colloid.	Macrofollicular adenoma and nodular goiter
Oncocytic variant	Papillary and follicular architexture, abundant coarsely granular cytoplasm with nuclear features of classical papillary carcinoma.	Papillary hurthle cell tumor – macronucleoli absent in oncocytic variant. Hashimotos thyroiditis.
Warthin tumor like variant	Oncocytic tumor cells, papillary strutures, brisk lymphoplasmacytic	Combination of Papillary carcinoma and hashimotos thyroiditis-

	infiltrate in papillary stalks[83].	lymphocytes and plasma cells intermingle with the cell clusters, in hashimoto s thyroiditis.
Cribriform morular variant	Closely packed follicles,papillae, solid areas with islands of squamoid morules. Nuclear chromatin fine powdery with occasional grooves and inclusions.	
Adenoid cystic variant	Papilliform clusters, monolayered sheets, many nuclear grooves and inclusions. Light pink to purple hyaline globules surrounded by neoplastic cells.[84]	
Nodular facitis like stroma	Predominate stromal component, with bland spindle cells,irreguar in shape and size. Sparse epithelial groups shows features of papillary carcinoma.	
Tall cell variant	Solid areas,follicles and papillae lined by oxyphilic cells twice as tall as other than variants. Oxyphilic cells shows reddish or cyanophilic granular eosinophilic vacuolated cytoplasm with nuclear grooves and inclusions. [49,50]	
Columnar cell variant	Mixed papillary, follicular, solid growth.tall columnar lines the papillary and follicular cells, pseudostratified cells lining papilloglandular strutures that resemble respiratory epithelial cells. Nuclear grooves and inclusions are	

	usually not seen.[52]	
Diffuse scerlosing variant	Prominent fibrosis with papillary pattern many psammoma bodies with lymphoplasmacytic infiltrate. Squamous metaplastic cells will be present. [85,86]	Hashimoto s thyroiditis.
Solid /trabecular variant	Solid and trabecular pattern with irregular nuclear contours with few cells showing nucear groooves and inclusions.	
Medullary carcinoma	Plasmacytoid, spindle cell , small cell pattern with nuclei showing moderate anisonucleosis, uniform stippled chromatin , few scattered cells coarse red cytoplasmic granularity (MGG) seen.	Papillary carcinoma – medullary carcinoma with papillary variant shows true papillae, with intranuclear cytoplasmic inclusions seen. It shoud be differetiated from papillary carcinoma thyroid. Definite diagnosis requires ancillary studies.

SURGICAL PATHOLOGY

LESIONS	GROSS FINDINGS	MICROSCOPY FINDINGS	DIFFERENTIAL DIAGNOSIS
Graves disaese	the gland shows a mild to moderate symmetric diffuse enlargement , reddish ,has a consistency of pancreatic tissue.	Microscopically the follicles are hyperplastic with prominent papillary infolding , it may confuse with papillary carcinoma. The lesion lined by columnar with basally located hypercromatic nuclei , and a clear cytoplasm, that may contain fat and glycogen. The collid is pale, finely vacuolated with prominent scalloping. The stroma contains lymphoid aggregate	Papillary carcinoma
Nodular hyperplasia	Grossly , capsule may be stretched , multiple nodules with secondary change haemarrhage, calcification and cystic degeneration are common.risation	Microscopically varying appearances, some with large follicles lined by flattened epithelium, others have papillary projection, it may confuse with papillary carcinoma. Rupture of follicle may lead to a granulomatous reaction, prominent vascularisation	Papillary carcinoma Follicular carcinoma
Classical papillary carcinoma thyroid.	Average 1.5 to 3 cm in size. Lesions are firm ,white in colour with an invasive tumor. Lesional	cells arranged in complex papillae ,with central fibrovascular core. Cells lining the	Hyperplasia of thyroid follicle – may sometimes exaggerate into papillary

	calcification is seen. Necrosis may be seen. Cystic formation may be associated with it.	papillae shows nuclear stratification ,irregular nuclear membrane with clear nuclear chromatin ,eccentric placed nucleoli. Intranuclear grooves and inclusions paresent. Psammoma bodies may be present.	change.
Follicular variant of papillary carcinoma	Well circumscribed encapsulated nodule.	Encapsulated microfollicles and macrofollicles,capsular invasion or vascular invasion may be present. Cells lining the follicles shows scant to eosinophilic cytoplasm with nuclear features of classical papillary carcinoma.	Follicular adenoma / carcinoma.-
Papillary microcarcinoma	Tumor less than 1 cm.	Nuclear features shows classical papillary carcinoma. Tumor is less than 1 to 1.5 cm.	
Tall cell variant	Large , infiltrative , white tan lesions. The tumor more than 6 cm .	Papillary growth pattern, cells showing cells height 2 to 3 times width with oncocytic cytoplasm. Focal to diffuse prominent nucleoli. Multiple intrnucear inclusions present. Vascular invasion and extrathyroidal extension more common.	Hurthle cell tumor- oncocytic cells may simulate tall cell variant.
Columnar cell variant	Tumors more than 6 cm. encapsulated or	Papillary pattern, lined by tall	

	circumscribed tumor.(brucen, thompson.)	columnar cells , nuclei are hyperchromatic , with punctate chromatin. Extrathyroidal extension most common.	
Warthin like variant.	Solid infiltrative ,ill defined tumor.	Paillary structure with cells showing abundant eosinophilic cytoplasm ,papillary core contains lymphoplasmacytic infiltrate.	Hashimotos thyroiditis and hurthle cell carcinoma.
Papillary thyroid carcinoma with nodular fascitis like stroma		Cells arranged in cords tubules and papillae with nuclear features of classical papillary carcinoma. Also shows varying degree of squamous metaplasia.	Anaplastic differentiation of papillary thyroid carcinoma.- in this spindle cell positive for cytokeratin. In nodular facitis spindle cell negative for cytokeratin.
Cribriform morular variant of papillary carcinoma.	well-circumscribed, somewhat lobulated tan masses ranging from 1.5-2 cm.	Cribriform ,solid and spindle cell growth pattern with nuclear features of papillary carcinoma.	
Diffuse scelerosing variant	Diffuse growth with fibrosis	Tumor cells lined by papillae, with psammoma bodies. Lymphocytic infiltrates around tumor foci.	
Hobnail variant		Tumor cell nuclei located in the middle or apex of cytoplasm.	
Encapsulated variant	Encapsulated tumor. Look like a adenoma groosly.	Microscopically shows total encapsulation with nuclear features and	

		psammoma bodies.	
Hurthle cell variant	Encapsulated,mahogany brown in colour.	) Papillary structures at least focally, may be mixed with follicular growth pattern.) Predominantly oncocytic cytoplasm but papillary nuclear features.	Hurthle cell tumor –benign or malignant.- it shows papillary areas.
Medullary carcinoma thyroid	Circumscribed, infiltrative tumor. Also admixed with necrosis and haemmorrhage.	Typical medullary carcinoma shows nests of tumor cells shows, composed of round to oval nuclei or spindle shaped cells.papillary variant of medullary carcinoma shows papillary architexture ,it may be pseudopapillary or true papillary .	Papillary carcinoma thyroid.

IMMUNOHISTOCHEMISTRY APPLICATION IN PAPILLARY LESIONS:

Immunohistochemistry is the application of immunologic principles and techniques to demonstrate specific antigens in cells and tissues based on antigen antiody interaction and it explore the specificity at light microscopic level.

Various methods of immunohistochemistry include eroxidase –antiperoxidase method, alkaline phosphatase labelling method, avidin biotin method, and two layer dextrin polymer technique.

STEPS OF IMMUNOHISTOCHEMISTRY:

Antigen retrieval:

Antigen retrival is done to unmask the antigen determinants of fixed tissue sections. This can be done by

1. Proteolytic enzyme digestion
2. Microwave antigen retieval
3. Microwave and trypsin antigen retrieval
4. Pressure cooker antigen retrieval [87]

Proteolytic enzyme digestion

Enzymes like trypsin and proteinases are used to breakdown the formalin cross linkages and unmask the antigen determinants. But there is a disadvantage of antigen destruction and inadequate digestion.

MICROWAVE ANTIGEN RETRIEVAL

In this formalin fixed paraffin sections are boiled in varoius buffers for rapid and uniform heating. This is the most common method used now.

PRESSURE COOKER ANTIGEN RETRIEVAL:

In this method also the tissue sections are boiled in buffers to unmask the antigens . this method is used to retrieve large number of slides.

DETECTIONS SYSTEMS:

After adding specific antibodies ,the antigens, the antigen antibody complex should be detected. This done by direct and indirect methods.

DIRECT METHOD:

The primary antibody is directly conjucated with flurochrome. Commonly used flurochromes are horse radish peroxidase and alkaline phosphatase.

INDIRECT METHOD:

It is a two step method. First the labelled secondary antibody reacts with primary antibody which is bound to specific antigen. The use of peroxidase enzyme complex or avidin biotin complex further increases yhe sensitivity of immunohistoc

Immunostaining shows that most papillary cancer contains thyroglobulin and thyroid transcription factor -1 .

A number of markers used for the differential diagnosis of thyroid lesions ,such are CD 56, HBME 1, CK 19, Galectin 3.

RET

The Ret gene is located on chromosome 10 q and encodes a tyrosine kinase transmembrane receptor. It is typically absent in the normal thyroid follicular cells; however, gene rearrangement occurs in most PTCs. This oncogene is believed to be specific to PTC and encodes an oncoprotein product that contains the cytoplasmic portion of Ret gene. Therefore, some investigators believe that IHC expression of Ret oncoprotein is a reliable marker for PTC . Cheung et al showed immune expression of Ret in 78% of PTC, 63% of FVPC and 57% of Hurthle cell carcinoma, while all benign nodules were non-immuno reactive for Ret. In our study, Ret was positive in 18/20 cases of PTC, in 45/54 (83.3%) of all carcinomas, and in 30/98 (30.6%) benign lesions.[88] Rossie et al

found that Ret had focal or moderate immune reactivity in benign lesions while it showed prevalent cytoplasmic expression in classic papillary carcinoma and its variants. The general conclusion among researchers is that diffuse immune expression represents a good supportive evidence for the diagnosis of papillary carcinomas; however, focal staining is often found in other lesions including benign nodules. Perhaps, a more important finding is that immune reactivity of a panel that includes Ret, HBME-1 and CK19 is very specific for papillary carcinoma. Cheung et al concluded that HBME-1 positivity indicates malignancy, whereas diffuse CK19 and/or Ret positivity confirm papillary differentiation .[88]

THYROID TRANSCRIPTION FACTOR 1

Thyroid Transcription Factor 1, also named NKX2 homeobox 1 (NKX2.1), is a nuclear protein, approximately 38 kDa, composed of a single polypeptide of 371 amino acids belonging to the family of homeodomain transcription factors. Thyroid transcription factor 1 plays a crucial role in the organogenesis and differentiation of thyroid and lung.

Thyroid transcription factor 1 expression by immunohistochemical analysis was initially exclusively identified in thyroid and lung epithelial tissues, including normal, benign, and malignant tissues. In routine practice, TTF1 became one of the most commonly used immunomarkers to identify thyroid or lung primary tumor in the setting of metastasis and to differentiate adenocarcinoma from squamous cell carcinoma in poorly differentiated non– small cell carcinomas of the lung in small biopsy or cytologic specimens. normal thyroid follicular cells

and parafollicular cells show diffuse expression of TTF1. In thyroid neoplasm, TTF1 expression was reported in nearly 100% of PTCs, FTCs, and follicular adenomas (FAs); in approximately 90% of papillary carcinomas and Medullary carcinomas; and in none to fewer than 25% of undifferentiated carcinoma.[89]

CD 56

CD 56 is a neural cell adhesion molecule . its expression may affect the migratory capability of tumor cells. Loss of CD 56 correlates with metastatic potentials and poor prognostic outcome in some malignancies. The marker pattern and intensity of staining were scored. Positive expression of the markers in 10% or more of follicular epithelium within the tumor or lesional cells was considered positive. An expression of <10% was considered to be negative. Diffuse CD56 expression was consistently present in normal, lesional, and neoplastic follicular epithelium, except for PTC, including the follicular variant. We concluded that CD56 is of value to distinguish PTC from other thyroid follicular pathology/histology with a sensitivity of 100% and a specificity of 100%. We suggest that CD56 is extremely useful in the diagnosis of PTC, including the follicular variant, and to distinguish it from other follicular cell-derived thyroid tumors/lesions. Application of CD56 by a group of expert pathologists on a larger series of follicular thyroid neoplasms of uncertain malignant potentials may potentially provide an objective diagnostic tool.[90]

HBME 1

HBME-1 is a monoclonal antibody that was initially promoted as a marker of mesothelial cells;[91] it is directed against an unknown epitope. In the thyroid, HBME-1 is almost exclusively expressed in malignant neoplasms, including papillary carcinoma, whereas benign lesions are negative. HBME-1 is the most specific marker of thyroid malignancy, but it may not be very sensitive because oncocytic lesions are generally negative; also, many malignancies are not stained by this antibody. HBME-1 positivity is characterized by predominantly membranous staining with variable cytoplasmic staining.

GALECTIN 3

It is a component of the beta galactoside binding lectin , function is not clear. It is involved in cell –cell and cell –matrix modulation. It is play a role in the malignant transformation of thyroid cells, expressed more in carcinomas, especially of the papillary type. [92]

P 63

p63, a p53-homologue nuclear transcription factor that is located on 3q27 and encodes six different isoforms, P63 tumor suppressing properties are disputable and mutations in this gene are rare in human malignancies . It is consistently expressed in basal, squamous and myoepithelial cells such as in basal cells of the prostate acini and ducts, myoepithelial cells of the breast and squamous cell carcinoma .[93]

E-CADHERIN

Cadherins are cell-cell adhesion molecules involved in the morphogenesis of developing tissues and maintenance of adult solid tissues. Cadherins binds to catenins that it have an intracellular domain . Loss of cell-cell adhesion is a major hallmark that correlates with loss of differentiation and aggressive tumor behavior. Normal thyroid follicular cells express uniformly high levels of E-cadherin mRNA and have a strong cell surface pattern of staining. E-cadherin staining is variably reduced in well-differentiated thyroid carcinomas and frequently absent in poorly differentiated and anaplastic carcinomas. loss of E-cadherin expression is an adverse prognostic factor in differentiated thyroid carcinoma.[94]

ROLE OF CK 19 IN PAPILLARY LESIONS OF THYROID

CK 19

Cytokeratin polypeptide 19 (CK19) is a type I intermediate filament protein . it is the smallest known keratin and remarkable in that, it is contrary to all other keratins because it does not have a designated partner for the formation of filaments, implying that regulation of its expression . Cytokeratin 19 concentrate at sarcomeres of striated muscle and combine with the dystrophin glycoprotein complex, through the interaction of the cytokeratin with the actin binding domain of dystrophin. In vitro studies showed that dystrophin binds directly and specifically to CK19 . CK19 is synthesized in simple and stratified epithelia. Strong and diffuse staining positivity in all cases of papillary

carcinoma. : confirm diagnosis of papillary thyroid carcinoma in cytology or equivocal cases , help distinguish follicular variant of papillary thyroid carcinoma (CK19+) from (a) follicular adenoma, trabecular adenoma, Grave's disease (weak / negative CK19,multinodular goiter with papillary areas. One study shows that, evaluation of CK-19 expression in neoplastic thyroid tissues, on the other hand, has proven useful in confirming diagnoses, when cytomorphology per se does not offer conclusive evidence. Positive immunocytochemical reactivity for CK19 was identified in 34 of 37 papillary carcinomas and in 1 of 36 other thyroid lesions (sensitivity of 92% and specificity of 97%). Although the strongest reactivity was obtained in methanol fixed thin layer preparations, the antibody also was effective in detecting papillary carcinoma in alcohol fixed and air-dried smears. The single false-positive case was a follicular adenoma with focal areas of papillary hyperplasia. All other aspirates including those from cases of Hashimoto thyroiditis, multinodular goiter, follicular adenoma, oncocytic neoplasms, and follicular carcinoma were negative. [95] It shows cytoplasmic and membranous positivity of cells.

Semi quantitative scoring of CK19 expression according to the percentage of positively stained cells[96]

Percentage of positively charged cells	**Extent of ck 19 positivity**
Nil	0
<5%	1+
5- 25%	2+
25 -75%	3+
>75%	4+

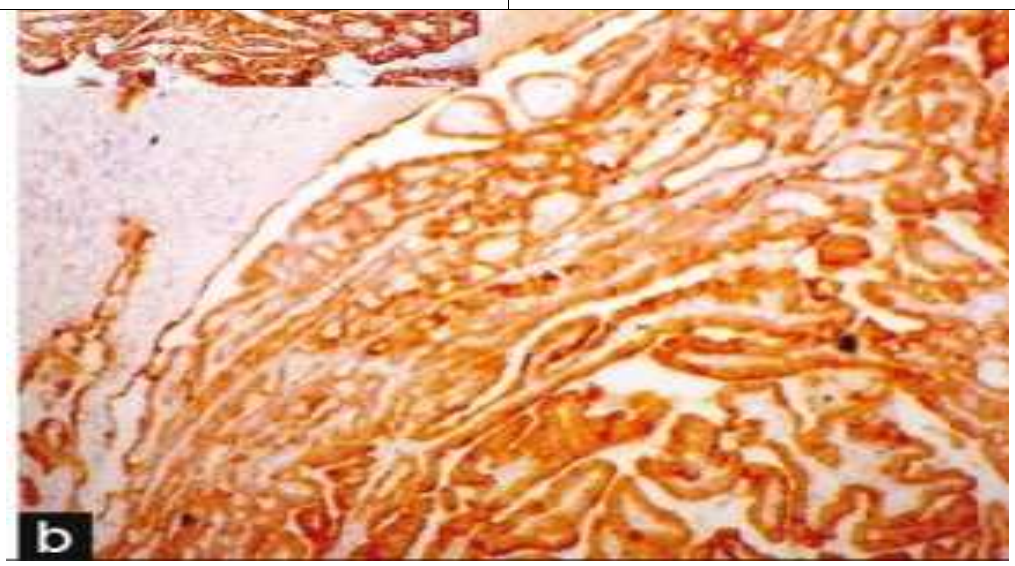
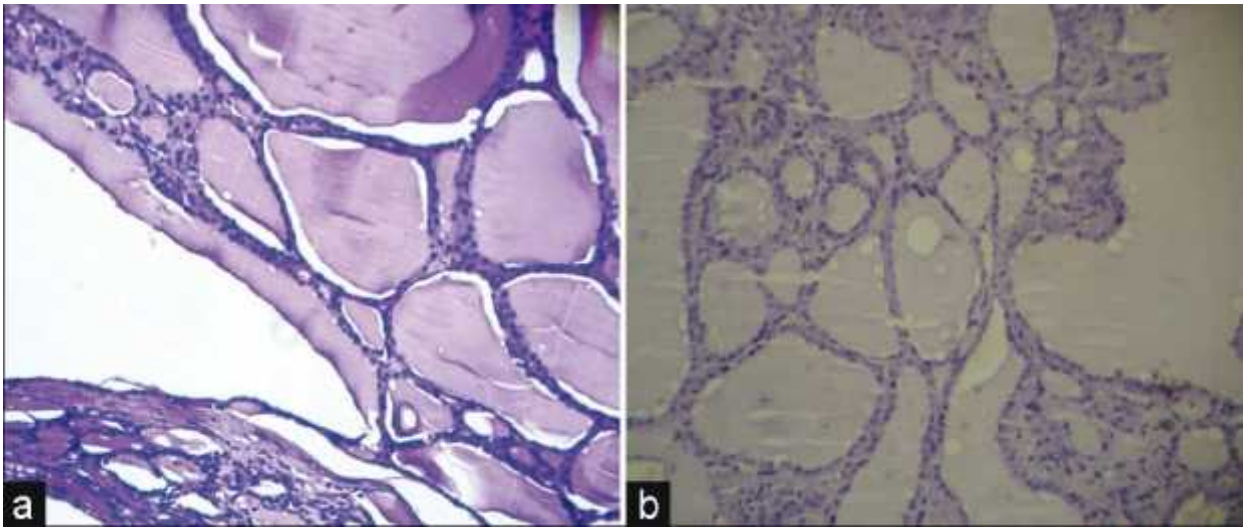


Figure 2 ck 19 expression in papillary carcinoma[96]



Multinodular goiter - negative staining.

Fig 3 multinodular goiter ck 19 expression[96]

Other study shows, All 22 (100%) papillary carcinomas showed diffuse and strong (3+ and 4+) CK19 expression. Six out of eight (75%) FAs and four out of eight (50%) MNG were positive for CK19, but it was of weaker intensity (1+ and 2+) and focal in distribution.

Focal CK19 staining may be found in benign disease, but diffuse and strong positivity is characteristic of PTC, which can be used in the diagnosis of PTC in lesions of equivocal morphological appearances.[96]

MATERIALS AND METHODS

STUDY LOCATION:

The study was undertaken in the Department of Pathology, Tirunelveli, Medical College.

STUDY PERIOD:

The study was conducted from the years 2015 to 2017.

SAMPLES:

A total of 46 cases including 29 cases of papillary carcinoma thyroid, 17 cases of papillary hyperplasia.

INCLUSION CRITERIA:

1. Papillary carcinoma and its variants
2. Beningn lesions with papillary hyperplasia

EXCLUSION CRITERIA:

1. Other malignancy including follicular carcinoma, medullary carcinoma without papillary areas.
2. Benign lesions without papillary hyperplasia

Materials required:

(1) Donor blocks which contains formalin fixed paraffin embedded tissue obtained from all the cases of papillary lesions of thyroid.

(2) Hematoxylin and eosin stained tissue sections made from the donor blocks.

(3) Postively charged slides for holding tissue sections for IHC

(4) Chemicals for preparing antigen retrieval solutions and for wash buffers

(5) microwave oven for antigen retrieval.

(6) Kit for performing immunohistochemistry which includes primary antibodies ck 19 and universal kit.

(7) Microscope used for grading of IHC slides

METHODOLOGY:

DATA COLLECTION:

The data including patients age, clinical status, and other clinical data were obtained from the pathology records.

PROCESSING OF SPECIMEN:

Total thyroidectomy and subtotal thyroidectomy specimens were received with 10% formalin and it is fixed for 24 hrs. section were taken carefully.

STAINING TECHNIQUE:

Sections of 4-5µ thickness were cut and stained with Haematoxylin & Eosin.The slides were studied under light microscopy and the data recorded.

HAEMATOXYLIN AND EOSIN TECHNIQUE:

PREPRATION OF HAEMATOXYLINE SOLUTION:

Haematoxyline 2.5 gm

Mercuric oxide 1.25 gm

Potassium alum 50 gm

Absolute ethyl alchol 125 ml

Sodium iodate 0.5 gm

Distilled water 500 ml

PROCEDURE:

Potassium alum ,50 gm is dissolved in 500 ml of distilled water by heating and shaking at 600C. Add solution of 2.5 gm of haematoxyline in 25 ml of absolute ethyl alchol and bring rapidly to boil. When to begins to boil, remove from flame and add 1.25 gm of mercuric oxide or sodium iodate. Mix by swirling gently.

PREPRATION OF EOSIN SOLUTION:

Eosin Y 1gm

95% ethanol 80 ml

Glacial acetic acid 0.2 ml

Distilled water 20 ml

PROCEDURE:

Dissolve 1gm of eosin y in 20 ml of distilled water and add 8o ml of ethanol and 0.2 ml of glacial acetic acid.

STAINING PROCEDURE

1.Xylene 3 changes -2 mins each

2.90%, 80%, 70% alchol each 5 min

3. Water wash 10 min

4. Harries haematoxyline 10 min

5. water wash 1o min

6. 1% Acid alchol 2 dip

7. tap water for blueing 10 min

8. 1% eosin 4 dips

9.water wash 5 min

10. acending grades of alchol 3 changes

11. xylene 2 changes

15. mount in DPX mount

After that screened the slide under light microscope , and papillary carcinoma and its variants and benign lesions with papillary hyperplasia were taken for IHC.

IMMUNOHISTOCHEMICAL EVALUATION:

Immunohistochemistry was performed on 3-4m-thick sections taken on poly-L-lysine-coated slides. Antigen retrieval was performed by heating the sections in tris-EDTA buffer at pH 6.0 using pressure cooker. Mouse Monoclonal antibody was used to bind with the primary antigen and is detected by adding secondary antibody conjugated with horse radish peroxidase – polymer and diaminobenzidine substrate. In this study , ck 19 antigens of Pathnsitu laboratory products is used.

PRECAUTIONS :

1.The glassware used should be dry and clean.

2.the buffer used should be prepared fresh and the ph should be adjusted according to preffered PH.

3. The staining procedures are never allowed to dry so they are performed under a humidity chamber.

4. DAB chromogen should be handled and disposed carefully as it is a carcinogen.

5. primary antibody ,DAB chromogen ,peroxidase block should be stored at 4-6 degree

6. then the slides are counterstained with haematoxyline.

BUFFER PREPRATION:

TRIS EDTA BUFFER

Tris –6.05gm

EDTA--O.744gm

1 N HCL—4ml

Distilled water—1litre

TRIS WASH BUFFER:

Tris – 6.05 gm

Sodium chloride- 8 gm

1NHCL- 4ML

Distilled water- 1000ml

PROCESSING FOR IMMUNOHISTOCHEMISTRY :

1. Cut 3mm sections on charged slides and incubate at 60-70C for 1 hour
2. Deparafinize by 2 changes of xylene 15 minutes each.
3. Hydrate through decending grades of alchol as follows

Absolute alchol two changes 5 minutes change

90% alchol 5 minutes

70 % alchol 5 minutes

Wash in distilled water , two changes ,2 minutes change.

4. Antigen retrival for 15-20 minutes in MERS , PH of retrival buffer may be either 6 ,8 or 9.5 according to the marker.
5. Wash in distilled water ,two changes ,2 minutes each.
6. Wash in PBS/ TBS for 2 minutes.
7. Endogenous peroxidase blocking by adding H2O2 on the sections, kept for 5 mins
8. Washed in wash buffer each 2 minutes.
9. Primary antibody were added and kept for 30 minutes then washed in wash buffer 2 times 2 minutes each.
10. Polyexcel target binder reagent added and kept for 12 minutes.
11. Washed in 2 changes of wash buffer.
12. Polyexcel HRP and incubate for 12 minutes.wash in wash buffer for 2 changes.
13. DAB chromogen added that is 1ml of DAB buffer, plus 1 drop DAB chromogen mixed well and kept for 2- 5 minutes.
14. Washed in distilled water for 5 minutes.
15. Counterstain with haematoxyline for 30 seconds, and washed in water.
16. Dehydrate through ascending grades of alchol.
17. Mounting is done by DPX mountant and observed under microscope.

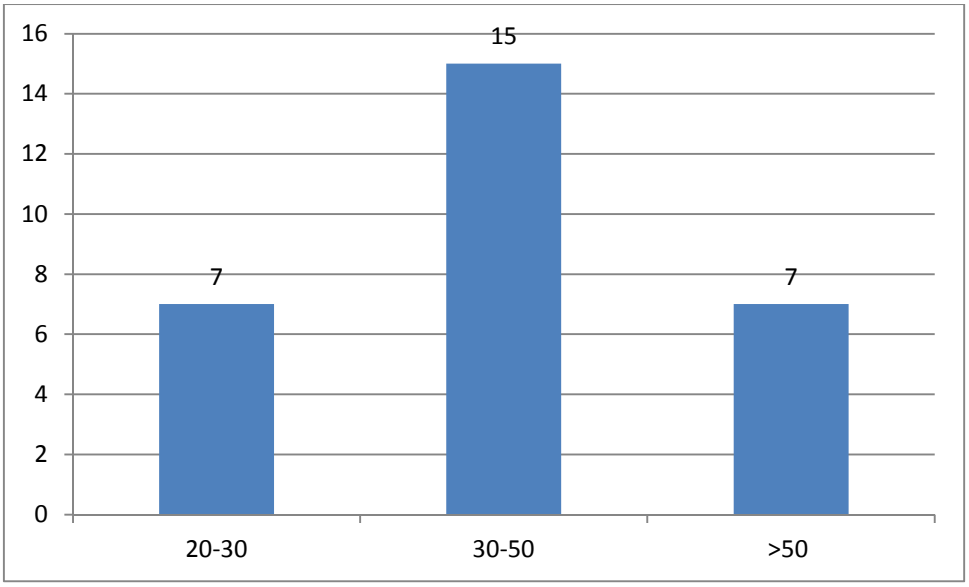
PERCENTAGE OF POSITIVELY CHARGED CELLS	EXTENT OF CK 19 POSITIVITY
Nil	0
<5%	1+
5- 25%	2+
25 -75%	3+
>75%	4+

Scoring of ck 19 is made by Semi quantitative scoring of CK19 expression according to the percentage of positively stained cells.

RESULTS AND STASTICAL ANALYSIS:

I AGE DISTRIBUTION IN PAPILLARY CARCINOMA:

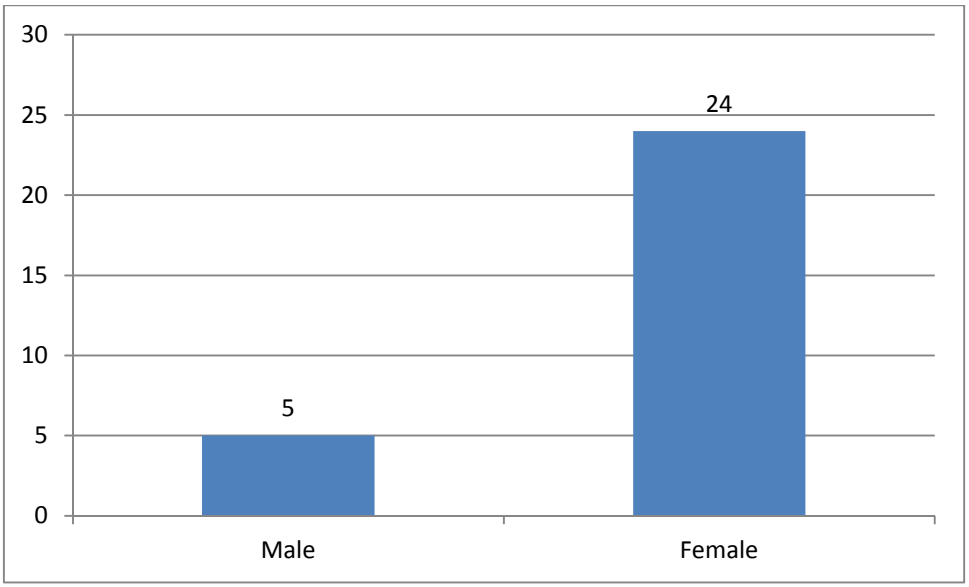
AGE GROUP	CLASSICAL PAPILLARY CARCINOMA	FOLLICULAR VARIANT OF PAPILLARY CARCINOMA	MICRO PAPILLARY CARCINOMA	INTRACYSTIC PAPILLARY CARCINOMA	COLUMNAR VARIANT PAPILLARY CARCINOMA
20-30 Years [1]	3	3		1	
30-50 years [2]	7	2	2	4	
Above 50 Years [3]	3	2		1	1



In this study most of the papillary carcinoma of thyroid occur in the age group between 20-50 years. Most of the cases were diagnosed mostly 3 rd to 5 th decades. In males , out of 29 cases 5 cases were males, mostly occur above 40 years. The mean age group is 43.9 years.

II SEX DISTRIBUTION:

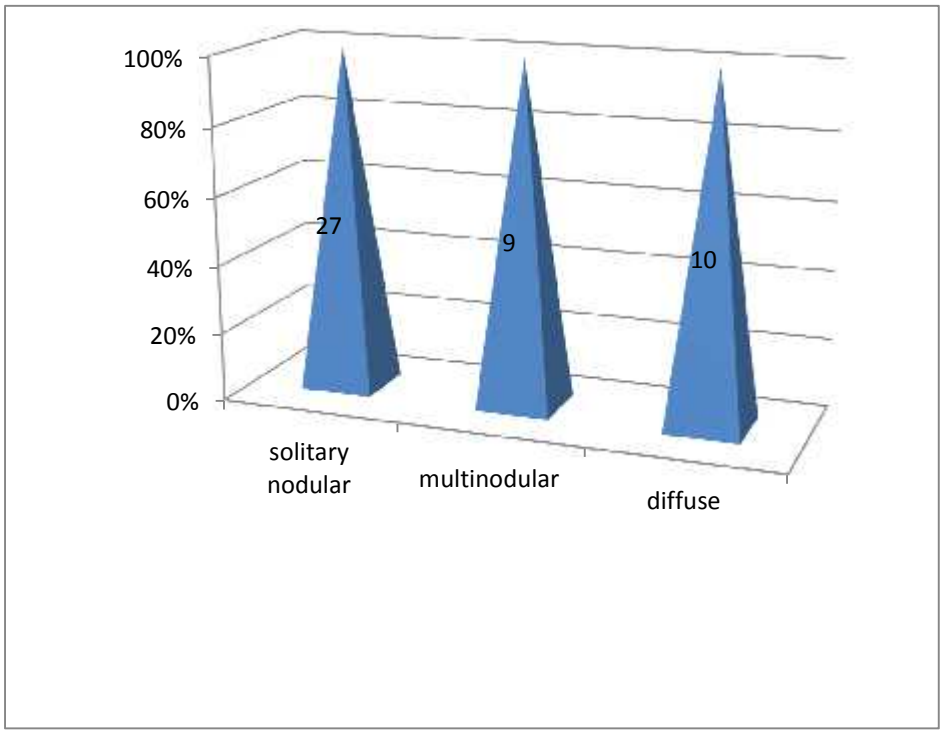
	classical papillary	Follicular variant	Micropapillary variant	Intracystic variant	Columnar cell variant
Male	2	1		2	
female	11	6	2	4	1



In this study 29 cases were papillary carcinoma, 17 cases were papillary hyperplasia, in papillary carcinoma out of 29 cases , 24 cases were females and 5 cases were males in the ratio of 1:8.

III FREQUENCY OF CASE PRESENTATION:

S.No	Clinical presentation	Frequency	Percent
1	Solitary nodular swelling	27	59
2	Multi nodular lesion	9	20
3	Diffuse lesion	10	22
	Total	46	100



In this study most of the papillary lesions arises from solitary nodular swelling , in our study out of 29 cases of papillary carcinoma 19 cases arises from solitary nodular swelling,5 cases from multinodular swelling , 5 cases from diffuse thyroid lesion.

		Frequency
diagnosis	Classical papillary carcinoma	13
	Follicular variant of papillary carcinoma	7
	Papillary microcarcinoma	2
	Intracystic papillary carcinoma	6
	Columnar cell variant	1
	Total	29

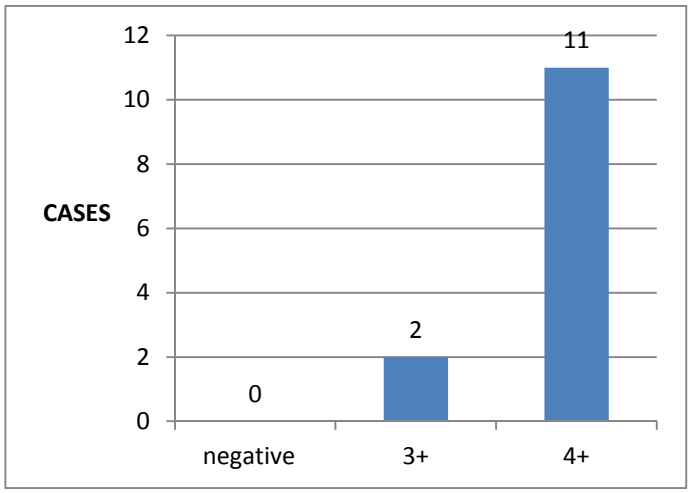
	Nodular goitre with papillary hyperplasia	**12**
Diagnosis	Hashimotos thyroiditis with papillary hyperplasia	**4**
	Graves disease with papillary hyperplasia	**1**

Out of 29 cases of papillary carcinoma 13 cases were classical papillary carcinoma, 7 cases were follicular variant of papillary carcinoma, 2 cases were micropapillary carcinoma, 6 cases were intracystic papillary carcinoma, 1 case were columnar cell variant.

In papillary hyperplasia, 12 cases were multinodular goitre with papillary areas, 4 cases were hashimotos thyroiditis with papillary areas, 1 case graves disease with papillary hyperplasia.

IV CK 19 EXPRESSION PAPILLARY CARCINOMA;

CLASSICAL PAPILLARY CARCINOMA:



In this study 13 cases were classical papillary carcinoma , Scoring of ck 19 was assesed by according to positivity of cells that is no cells staining graded as 0, <5 % of cells graded as 1, 5-25% graded as 2, 25 -75 % graded as 3, > 75 % of cells positivity graded as 4. In our study, ck 19 expression in out of 13 cases, 11 cases with 4+ positivity that is more than 75 % of cells positivity. 2 cases were 3 + Positivity.

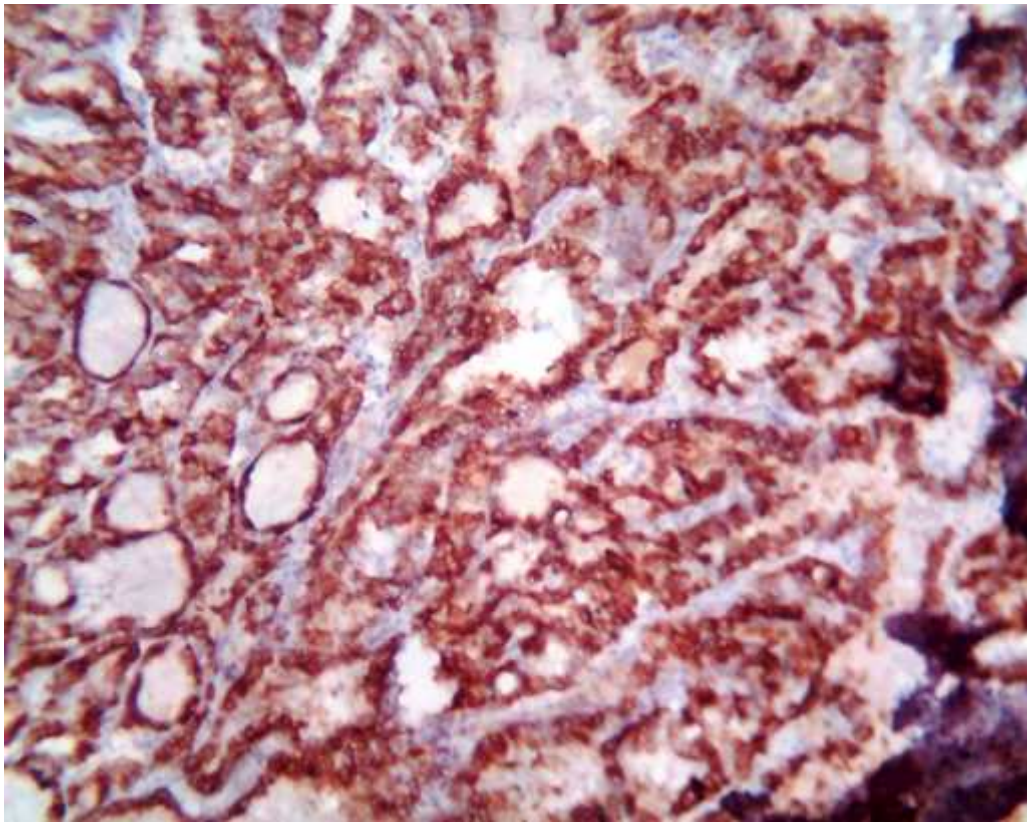
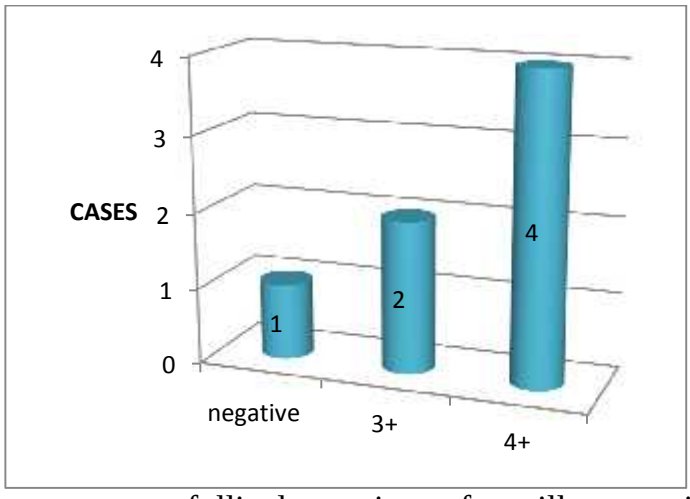


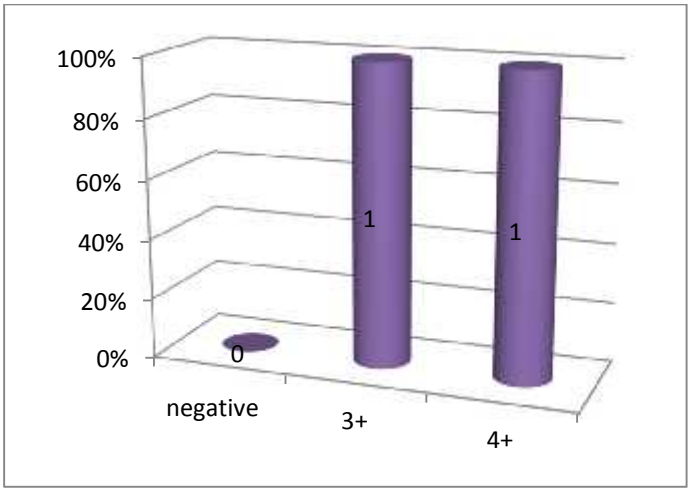
Fig 1. Papillary carcinoma shows diffuse positivity.

FOLLICULAR VARIANT OF PAPILLARY CARCINOMA:



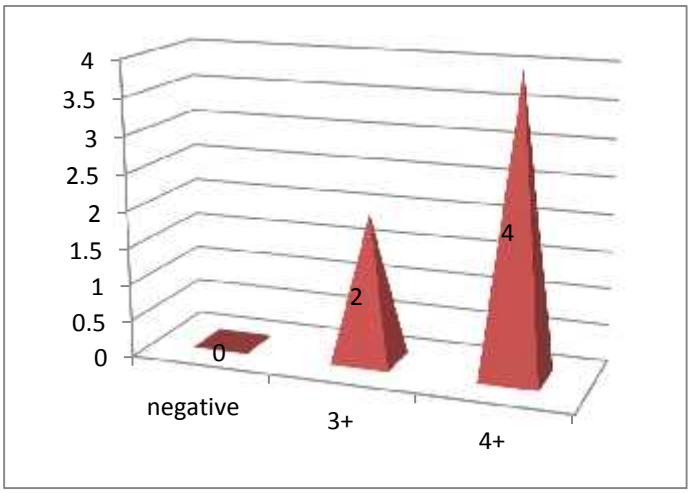
In this study 7 cases were follicular variant of papillary carcinoma , ck 19 expression in out of 7 cases, 4 cases shows 4 + positivity, 2 cases shows 3+ positivity, 1 case with 0. Negativity should take consideration.

MICROPAPILLARY CARCINOMA:



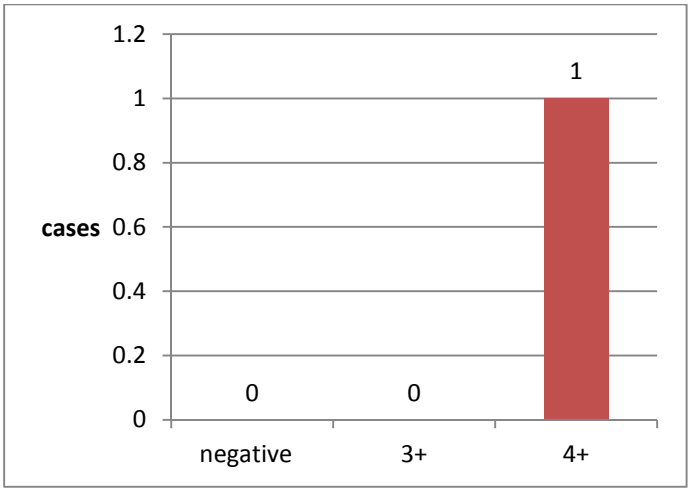
In our study 2 cases were, micropapillary carcinoma both cases show diffuse positivity 1 case with 4+, 1 case with 3+ positivity.

INTRACYSTIC PAPILLARY CARCINOMA:



In our study 6 cases were intracystic papillary carcinoma, 4 cases shows 4 + positivity, 2 cases with 3+ positivity.

COLUMNAR CELL VARIANT OF PAPILLARY CARCINOMA:



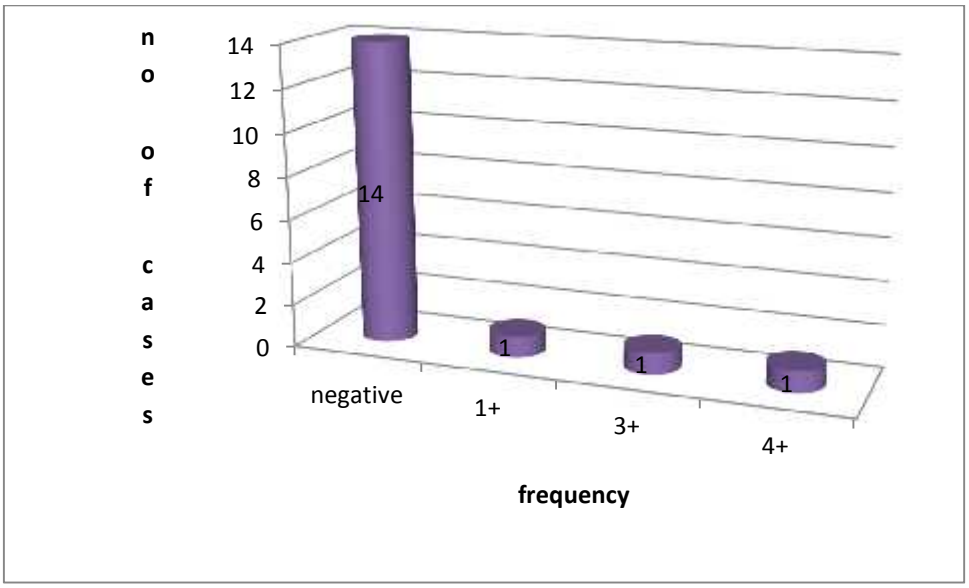
1 Case of columnar cell variant shows also diffuse cytoplasmic 4 + positivity.

		CK19			P value
		negative	3 +	4+	
Diagnosis	Classical PTC	0	2	11	0.716
	Follicular variant	1	2	4	
	Micropapillary	0	1	1	
	Intracystic variant	0	2	4	
	Columnar cell variant	0	0	1	

In our study 29 cases were papillary carcinoma, out of 29 cases, 7 cases show 3+ positivity, 21 cases with 4+ positivity, 1 case of follicular variant of papillary carcinoma shows negativity. All variant of papillary carcinoma shows

diffuse positivity. There is no significant difference between percentage of ck 19 expression in variants of papillary carcinoma.

PAPILLARY HYPERPLASIA:



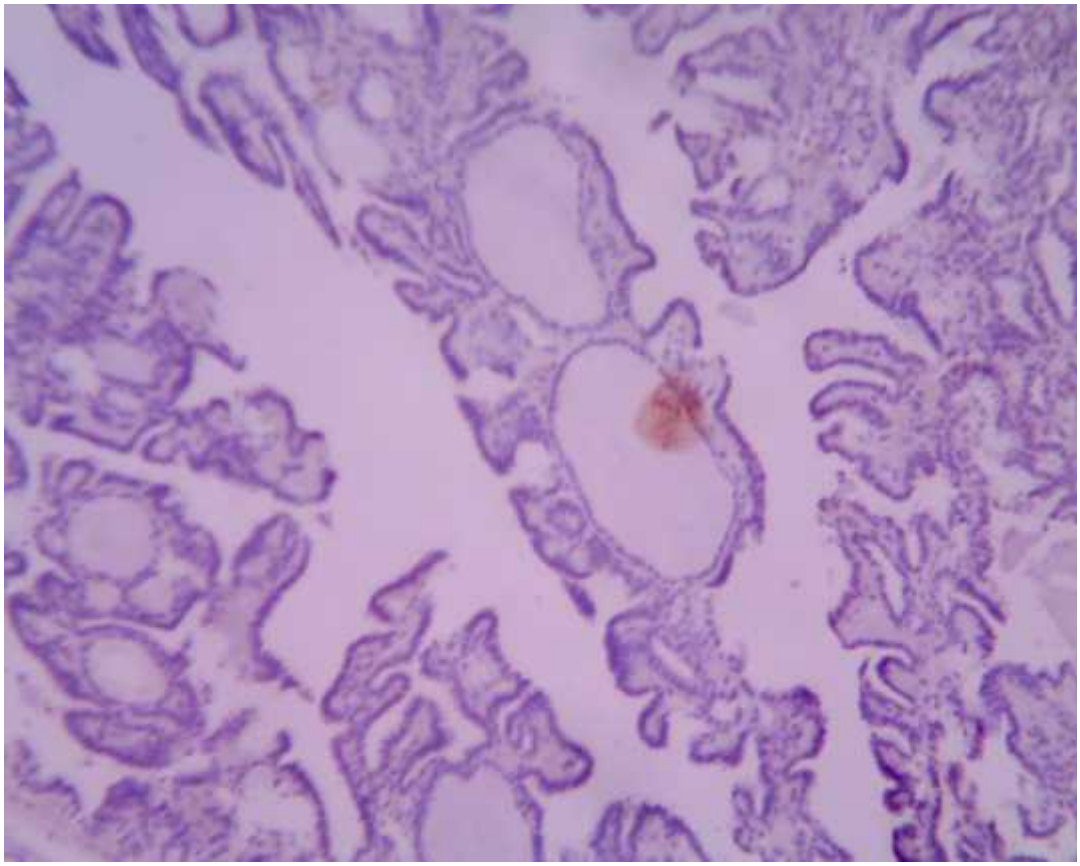
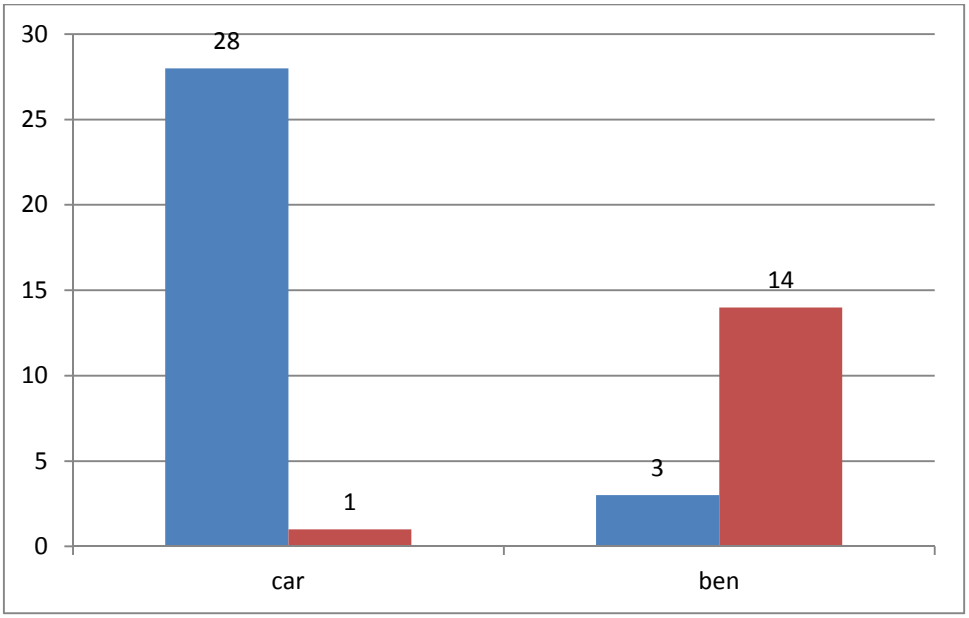


Fig 2 papillary hyperplasia in Multinodular goitre shows negative stain.

In our study , 17 cases were papillary hyperplasia, 12 cases were multinodular goitre with papillary hyperplasia, 4 cases were hashimotos thyroiditis with papillary hyperplasia, 1 case of graves disease with papillary hyperplasia. In our study ,out of 17 cases, 14 cases show negative, one case with intracystic hyperplasia shows diffuse positivity, 2 cases with hashimotos thyroiditis shows positivity, out of 2 cases, 1 case shows focal positivity in reactive areas but not in papillary areas, few hurthle cells shows positivity. Other case shows diffuse positivity.

Comparison between papillary carcinoma and papillary hyperplasia:



	Positive	**Negative**	P value
Papillary carcinoma	**28**	**1**	<0.0001
Papillary hyperplasia	**3**	**14**	

In our study 29 cases were papillary carcinoma, 28 cases of papillary carcinoma shows diffuse positivity, 1 case reported as follicular variant of papillary carcinoma ,it was negative for ck 19. 17 cases were papillary hyperplasia, 14 case with papillary hyperplasia were negative. 3 cases were positive ,1 case shows focal positivity, that is few hurthle cells in reactive areas only shows positivity but not in papillary areas. One case with intracystic papillary hyperplasia shows difuse positivity , more than 80% of cells show positivity. There is a significant difference between papillary carcinoma and papillary hyperplasia in ck 19 expression.

SENSITIVITY AND SPECIFITY OF CK 19 EXPRESSION IN DIAGNOSTIC SIGNIFICANCE OF PAPILLARY LESIONS OF THYROID

Senstivity	Specificity	PPV	NPV	Likelihood ratio
93.33%	93.75%	96.55%	88.24%	38.42

In our study there is a high sensitivity and specificity in diagnostic value of ck 19 expression in papillary lesions of thyroid.

DISCUSSION

Papillary thyroid carcinoma (PTC) is the most common form of malignant thyroid neoplasm. Its diagnosis is based on nuclear features such as nuclear clearing, overlapping, grooves and pseudoinclusions. However, identification of these features remains, at times, difficult because of its focal presence and thus the distinction of Papillary thyroid carcinoma from other thyroid lesions may not be possible. There are several other thyroid lesions that may contain papillary processes with nuclear features in a focal manner, which pose diagnostic difficulties with Papillary thyroid carcinoma. Multinodular goiter (MNG) with delicate papillary budding and focal nuclear clearing may be confused with Papillary thyroid carcinoma. Several immunohistochemical stains have been investigated for their possible role as diagnostic markers for Papillary thyroid carcinoma. They are cytokeratin19 (CK19), HBME1, galectin-3 and RET and thyroid transcription factor 1. Although galectin-3 was initially shown to have utility in the differential diagnosis between benign and malignant thyroid lesions, recent studies suggest that it is not reliable. Several studies have shown conflicting results regarding the usefulness of CK19 as a diagnostic marker in PTC.This study was carried out to investigate the role of CK19 as a possible diagnostic marker of PTC and its utility in differentiating Papillary thyroid carcinoma, from the other benign thyroid lesions with papillary areas mimicking papillary carcinoma.

In our study, 29 cases were papillary thyroid carcinoma ,mostly occur in age range of 20 to 50 years with an median age is 43.9 years, in bose et al showed a mean age was 34.50 years. In our study it is slightly higher than that. [96]

Ikram et al studies showed most of the cases occur 25 to 55 years , in our study also most of the papillary carcinoma diagnosed in the age group between 20 to 50 years. [3] suna and abdullah et al shows mean age is 44.8 years it was similar to our study[97].

In this study male female ratio is 1:8 it is slightly more than already puplished literature. Bose et al showed the ratio is 1:6.3,slightly lower than our study.

Different studies have shown varying percentage distribution of ck 19 positivity in papillary carcinoma thyroid and other lesions of thyroid. In this study 29 cases were papillary carcinoma includes [13 cases were classical papillary carcinoma, 7 cases were follicular variant of papillary carcinoma, 2 cases were micropapillary carcinoma, 6 cases were intracystic papillary carcinoma, 1 case were tall cell variant of papillary carcinoma], 17 cases were other lesions with papillary hyperplasia.

Out of 17 cases of papillary hyperplasia , 11 cases were multinodular goitre with papillary hyperplasia,4 cases were hashimotos thyroiditis with papillary hyperplasia, 1 case of graves disease, 1case with intracystic papillary hyperplasia.

In this study all variants of papillary carcinoma were diffuse cytoplasmic and membranous positivity, one case reported as follicular variant of papillary carcinoma were negative for ck 19 expression. 21out of 29 cases shows 4+ positivity, 7 out of 29 cases shows 3+ positivity, 1 case shows negative expression.

Bose et al study showed CK 19 semiquantitative score was analyzed, 17 out of 22 (7 PTC showed 4+ positivity and the remaining five expressed 3+ positivity. All showed diffuse positivity. It was similar to our study.

Suna and ekrilic et al showed, Diffuse and intense cytoplasmic CK19 positivity was found in all 25 cases of papillary carcinomas (100%)[97] Hasan et al showed, Sixteen of the 20 papillary carcinomas showed diffuse and intense cytoplasmic staining with CK19 (80%), 4 cases showing diffuse faint staining (20%).[3]in our study all cases were diffuse positivity, only one case were negative. Negativity should take consideration, some studies revealed that negative stain good evidence against papillary carcinoma[96]

Barut et al studies showed, CK-19 was also focally expresssed in Hashimoto's thyroiditis and lymphocytic thyroiditis.[98] in our study also 4 cases were hashimotos thyroiditis with papillary hyperplasia , 2 cases were complete negative, 1 case with focal positivity in reactive hurthle cell areas, but not in papillary areas, but 1 case shows diffuse positivity, but why this positivity is not explainable. It should take consideration.

Hasan et al showed, Seven of the 10 Grave's cases (70%) are completely negative. The remaining 3 cases showing focal weak staining with CK19 (30%). There was a significant difference in the extent of staining between papillary thyroid carcinoma and Grave's disease and there was highly significant difference in intensity of staining between them . in our study 1 case were graves disese with papillary hyperplasia, shows also complete negativity.

Suna and ekrilic et al showed In the multinodular goiter group, 20 of 25 cases showed no staining while the remaining 5 were focally reactive with CK19. Three of the five were thought to be false positive owing to hemorrhage. Weak and focal CK19 staining was seen in some follicular adenomas. Our observations suggest that the staining features of CK19 may be helpful in differential diagnosis between papillary carcinoma and multinodular goiter showing papillary areas. Focal and pale staining for CK 19 may be seen in multinodular goiter with papillary formations, and this feature should be considered in evaluation. in our study 12 cases were multinodular goiter with papillary hyperplasia, 1 case of nodular goiter with intracystic papillary hyperplasia, 11 cases were negative for ck 19, 1 case with intracystic hyperplasia shows diffuse positivity, after that we took rebits from suspicious areas, and it turned to be a intracystic papillary carcinoma. In suna and ekrilic studied also 20 cases were negative, 5 cases were positive but in haemorrhagic areas only.[97] With that negative stain is a good evidence against papillary carcinoma. But positive stain against anti ck 19 antibodies should take consideration. We

conclude that ck 19 is a useful marker to differentiate papillary carcinoma from papillary hyperplasia in other benign lesions.

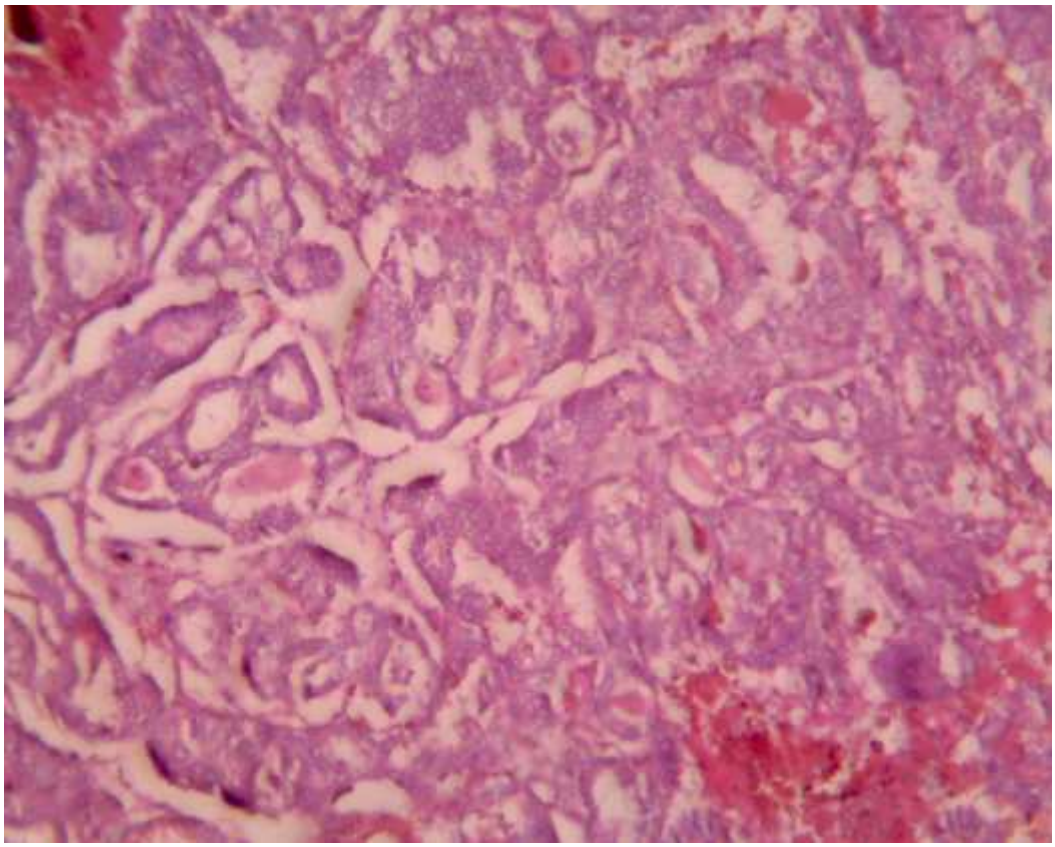
SUMMARY

- Thyroid malignancies are the most common endocrine malignancies. In that papillary carcinoma is the most common tumor of thyroid.
- Papillary carcinoma mostly diagnosed by its typical nuclear features such as ,nuclear crowding , overlapping, and intranuclear cytoplasmic inclusions.
- Some times, benign lesions of thyroid , such as nodular goitre, hashimotos thyroiditis, graves disease may show papillary areas, it may underdiagnose as papillaery hyperplasia.
- In our study , to evalute the usefullness of ck 19 in differentiating the papillary carcinoma and papillary hyperplasia.
- Papillary carcinoma shows a diffuse and strong cytoplasmic and membranous positivity.
- Papillary hyperplasia shows negative stains, but sometimes hurthle cells, some reactive areas can take positivity, positive stain should take consideration, careful evalution is essential. Negativity is a strong evidence against papillary carcinoma.

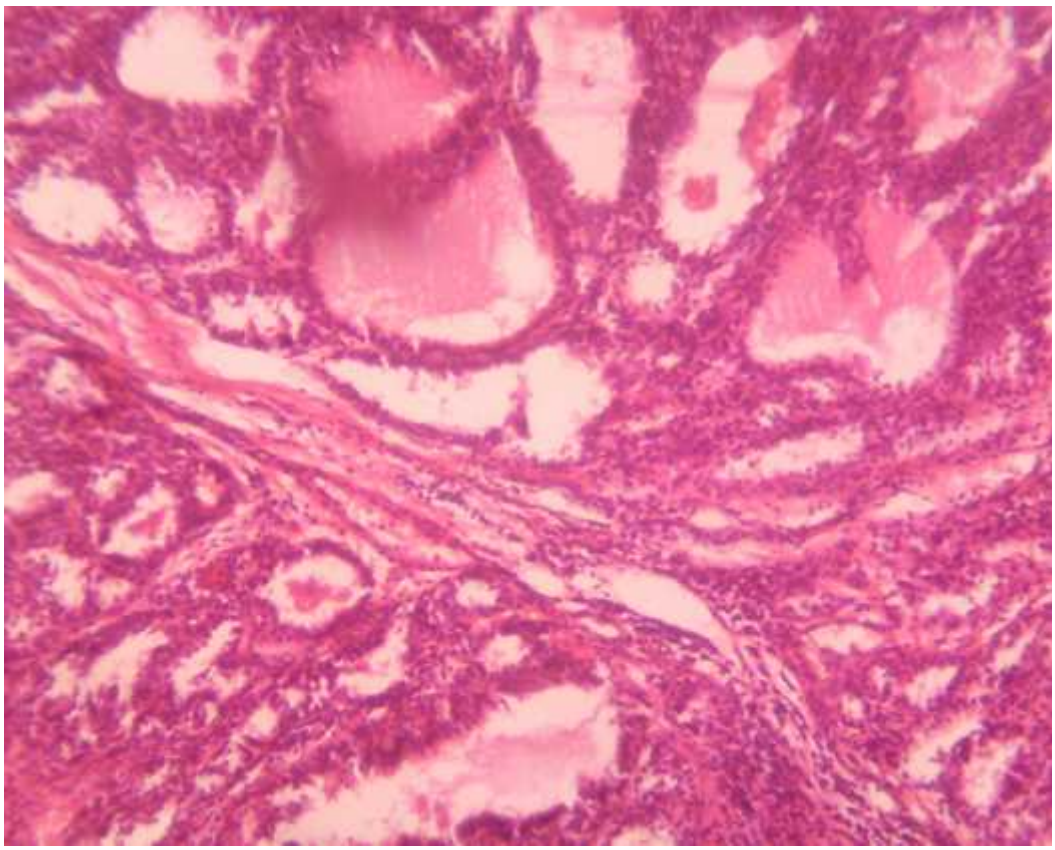
CONCLUSION

In our study most of all papillary carcinoma and its variants shows diffuse and strong positivity against anti ck 19 antibody. Papillary hyperplasia shows negative stain except 2 cases. We conclude diffuse and strong positivity confirms that the diagnosis of papillary carcinoma thyroid. Negative stain indicates it is a benign lesion. Cytokeratin 19 is a useful marker to differntiate papillary carcinoma from other benign lesions shows papillary hyperplasia.

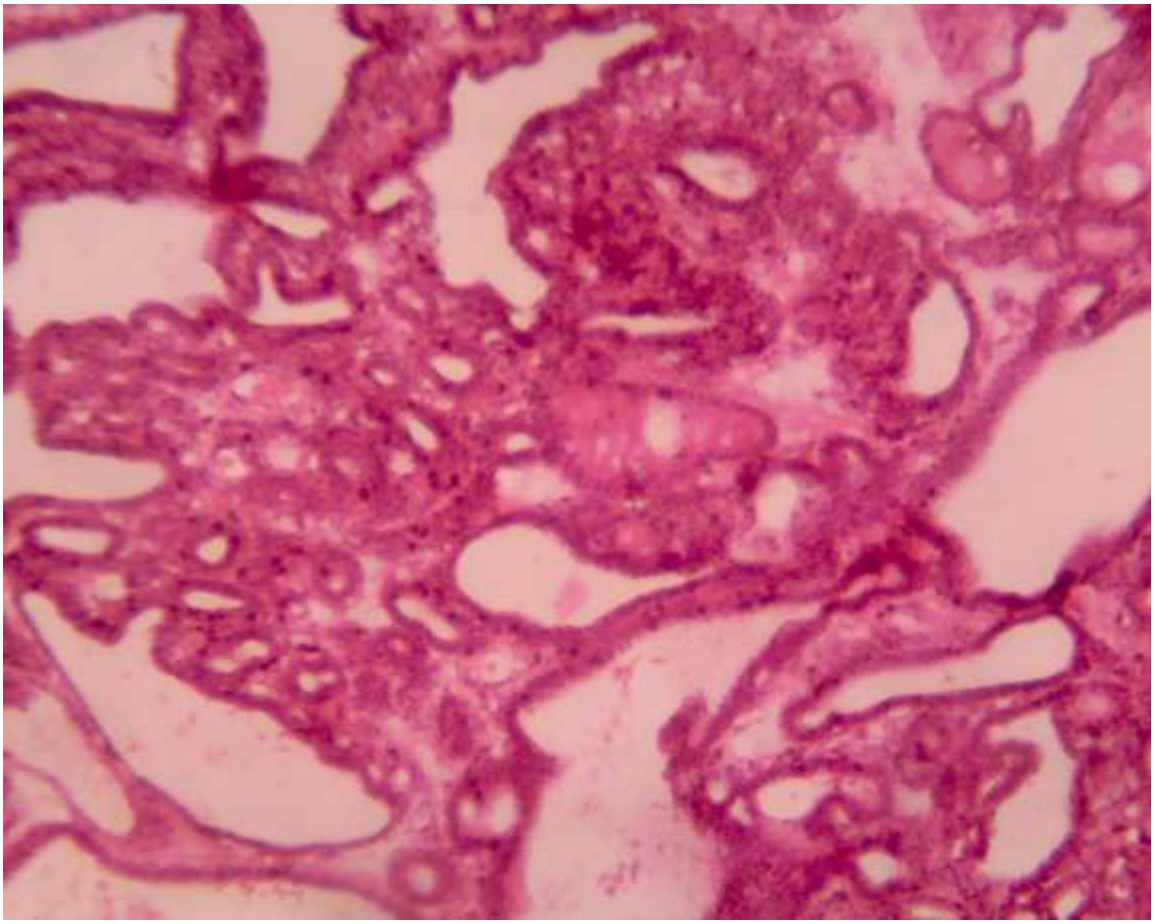
H & E IMAGE



INTRACYSTIC PAPILLARY CARCINOMA



GRAVES DISEASE WITH PAPILLARY HYPERPLASIA



NODULAR GOITRE WITH PAPILLARY HYPERPLASIA

IHC IMAGE

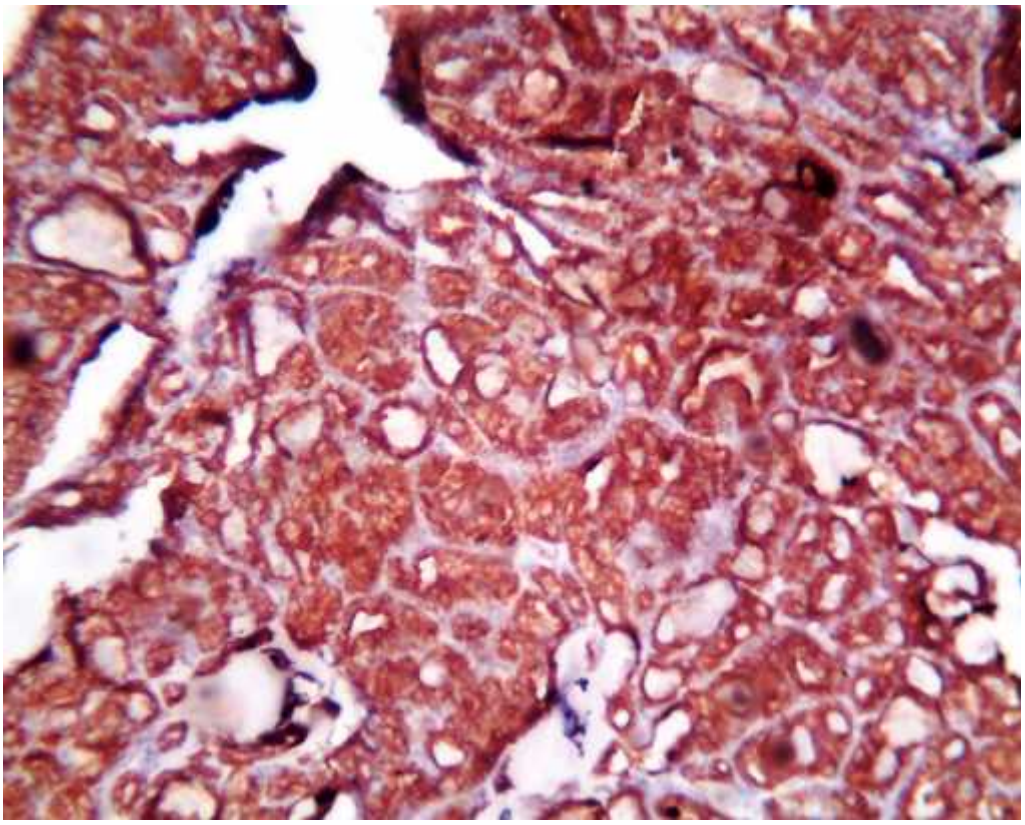
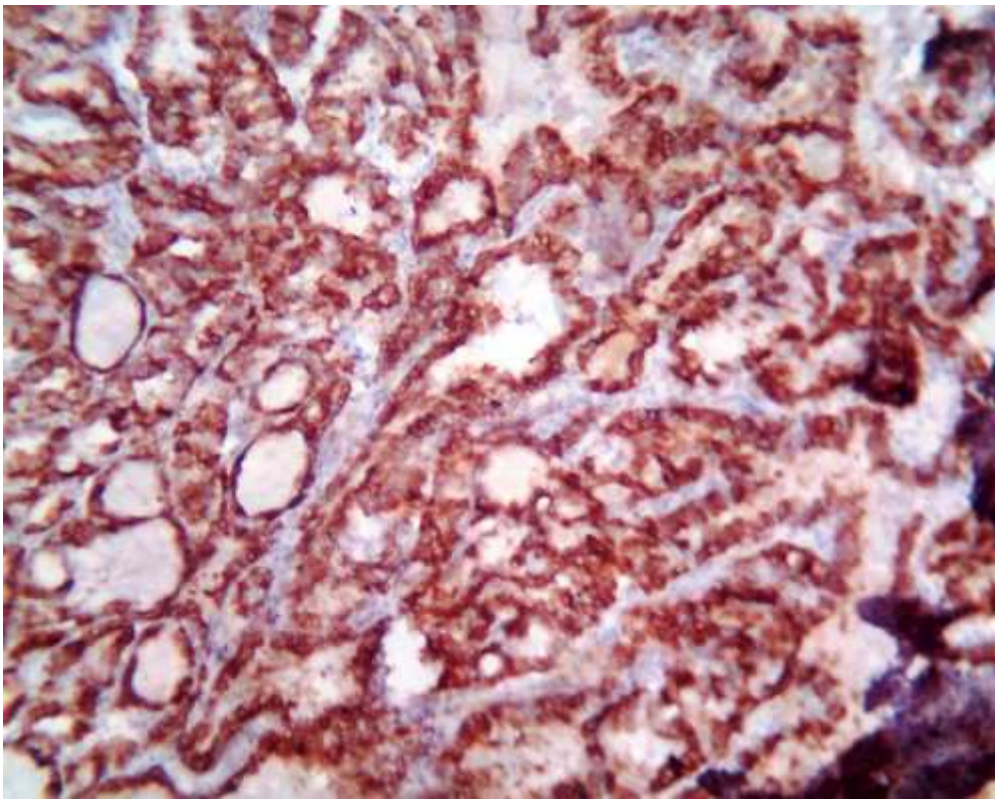
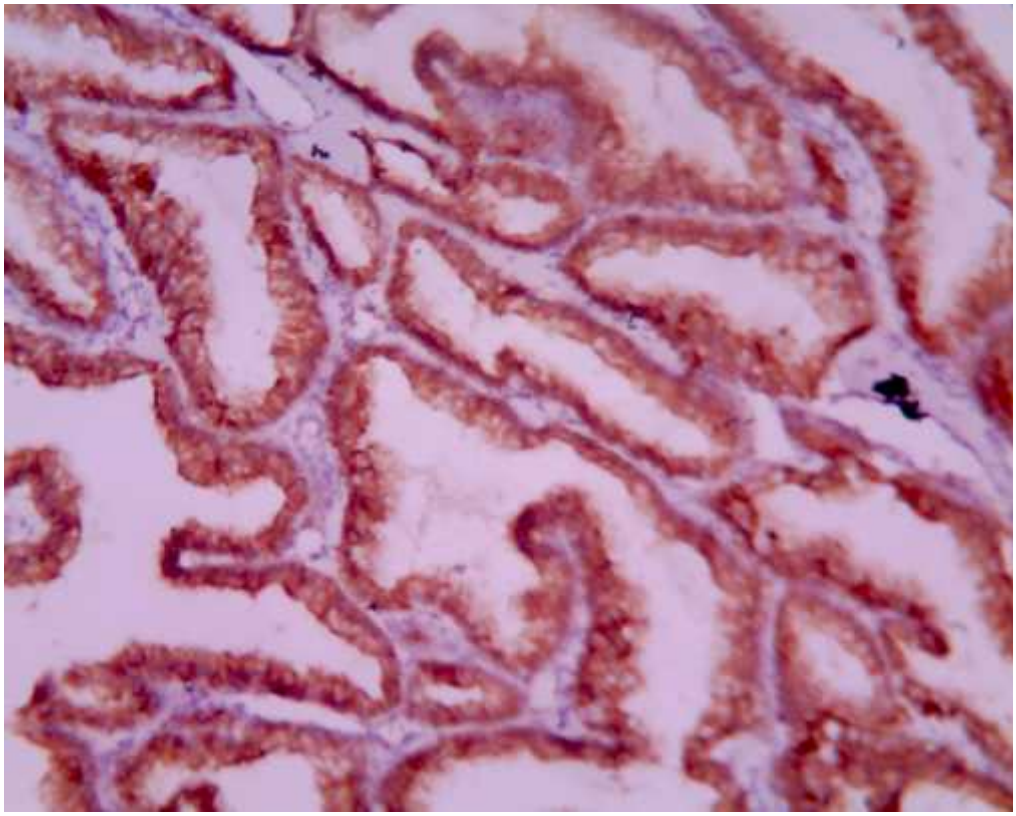


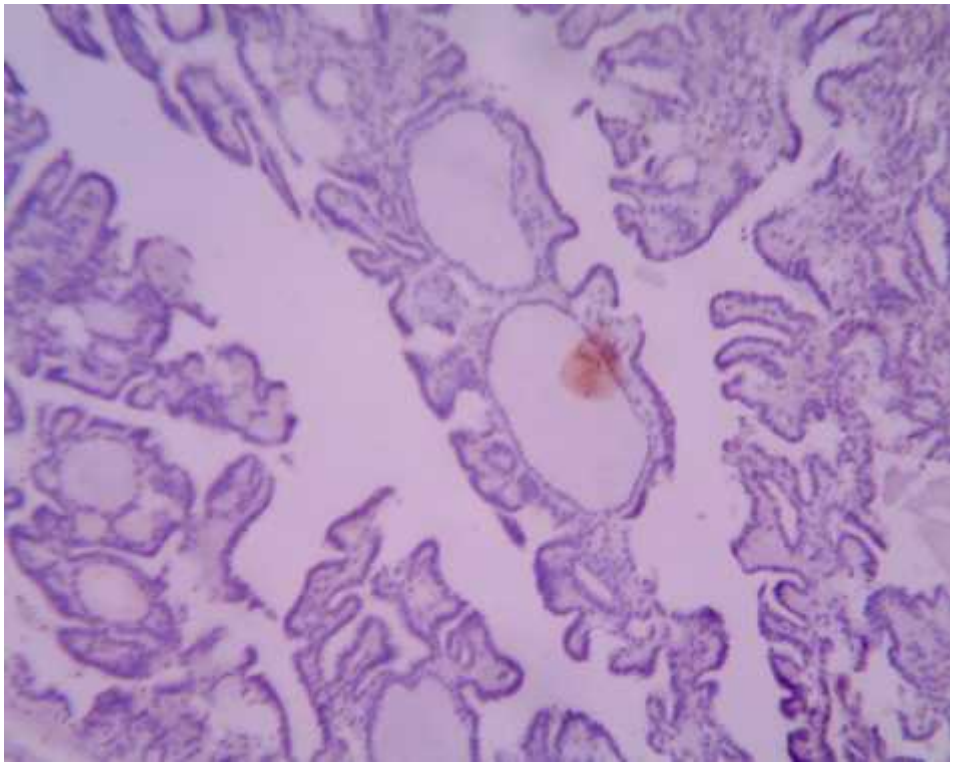
FIG 1 PAPILLARY CARCINOMA DIFFUSE POSITIVITY



INTRACYSTIC PAPILLARY CARCINOMA DIFFUSE POSITIVITY



COLUMNAR CELL VARIANT OF PAPILLARY CARCINOMA –DIFFUSE POSITIVITY.



GRAVES DISEASE WITH PAPILLARY HYPERPLASIA SHOWS NEGATIVE STAIN.

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SNO	AGE	SEX	IP NO	PATH NO	T3	T4	TSH	CLINICAL PRESENTATION	USG	FNAC	HISTOPATHOLOGY DIAGNOSIS	CK 19	% OF CELLS
1	21	F	10782	662/15	123.6	9.3	0.67	SNS	SOLITARY NODULAR SWELLING	NODULAR COLLOID GOITRE	INTRACYSTIC PAPILLARY CARCINOMA BACKGROUND COLLOID GOITRE	4+	100%
2	37	F	30765	1614/15	98.2	7.6	5.3	SNS	NODULAR LESION	NODULAR GOITRE	PAPILLARY CARCINOMA IN A BACKGROUND OF COLLOID GOITRE	4+	90%
3	30	F	43308	2345/16	75	11.6	1.7	SNS	SOLITARY NODULAR SWELLING	NODULAR GOITRE	PAPILLARY CARCINOMA	4+	100%
4	35	F	52771	3017/15	98	5.6	0.89	MNS	MULTINODULAR GOITRE	NODULAR GOITRE	INTRACYSTIC PAPILLARY CARCINOMA	4+	100%
5	66	F	37389	2036/15				MNS	MULTINODULAR GOITRE	PAPILLARY CARCINOMA	MULTINODULAR GOITRE WITH AREAS OF PAPILLARY HYPERPLASIA	NEGATIVE	
6	37	F	43043	2300/15	115	9.2	2.2	DTL	DIFFUSE THYROID LESION		PAPILLARY HYPERPLASTIC NODULE	NEGATIVE	
7	45	F	53368	2966/15	62	5.3	0.36	SNS	SOLITARY NODULAR SWELLING	NODULAR GOITRE	NODULAR GOITRE WITH PAPILLARY HYPERPLASIA	NEGATIVE	
8	65	F	58145	339/16	69.6	8.4	6.7	SNS	NODULAR LESION		PAPILLARY CARCINOMA	4+	100%
9	25	F	7123	382	66.6	11.3	7.2	DTL	DIFFUSE THYROID LESION	NODULAR COLLOID GOITRE	FOLLICULAR VARIANT OF PAPILLARY CARCINOMA	4+	90%
10	60	M	11728	655	76.9	7	3.3	SNS	SOLITARY NODULAR SWELLING	PAPILLARY CARCINOMA	INTRACYSTIC PAPILLARY CARCINOMA	4+	100%
11	47	F	12794	853	99.3	70.3	3.8	DTL	DIFFUSE THYROID LESION	NODULAR GOITRE	PAPILLARY MICROCARCINOMA WITH HASHIMOTOS THYROIDITIS	4+	80%
12	31	F	31009	1667/16	98	9.5	10.5	SNS	NODULAR LESION	HASHIMOTOS THYROITIS	FOLLICULAR VARIANT OF PAPILLARY CARCINOMA	4+	90%
13	47	M	32815	1870/16	151.4	10.5	O.74	MNS	MASS LESION WITH CERVICAL NODE	PAPILLARY CARCINOMA	PAPILLARY CARCINOMA WITH NODAL METASTASIS	4+	90%
14	50	F	41102	2255/16	1.41	5.87	30.8	DTL	DIFFUSE THYROID LESION	COLLOID GOITRE	MULLTIFOCAL PAPILLARY CARCINOMA WITH HASHIMOTOS THYROIDITIS	3+	70%
15	46	F	38390	2274/16	2.1	5.87	0.9	SNS	NODULAR LESION WITH COLLOID		FOLLICULAR VARIANT OF PAPILLARY CARCINOMA	3+	70%
16	25	M	44200	2447/16	153	7.6	9.3	SNS	COLLOID FILLED NODULE	NODULAR COLLOID GOITRE	FOLLICULAR VARIANT OF PAPILLARY CARCINOMA	4+	90%
17	50	F	53861	3035/16				SNS	NODULAR LESION	HAEMORRHAGIC CYSTIC NODULE	INTRACYSTIC PAPILLARY CARCINOMA	4+	90%
18	30	F	70890	3902/16	98.5	11.7	5.2	SNS	NODULAR LESION	PAPILLARY CARCINOMA	PAPILLARY CARCINOMA	4+	90%
19	52	F	75652	4223/16	77.3	8.2	5.3	SNS	MASS LESION	CYSTIC DEGENERATION IN NODULAR GOITRE	ION	4+	100%
20	46	F	83028	4564/16	112.3	9.3	3.8	DTL	DIFFUSE THYROID LESION		PAPILLARY CARCINOMA	4+	
21	37	F	20893	1257/15	1O9	6.8	3	SNS	COLLOID FILLED NODULE	NODULAR GOITRE	PAPILLARY CARCINOMA BACKGROUND OF COLLOID	4+	100%
22	35	F		1760/16	126	3.3	19.1	MNS		NODULAR GOITRE	PAPILLARY HYPERPLASTIC NODULE	NEGATIVE	
23	25	F		1762/16	112	6.7	9.26	SNS		NODULAR GOITRE	PAPILLARY HYPERPLASIA	NEGATIVE	
24	27	F	38991	2208/16	85	2.2	3.2	MNS	NODULAR LESION	NODULAR GOITRE	PAPILLARY HYPERPLASIA IN HASHIMOTOS THYROIDITIS	NEGATIVE	
25	38	F	51625	2844/16	88.2	7	9.3	DTL	DIFFUSE THYROID LESION	HASHIMOTOS THYROITIS	PAPILLARY HYPERPLASIA IN HASHIMOTOS THYROIDITIS	NEGATIVE	
26	51	F	50022	2847/16	152	7.6	11.6	DTL	DIFFUSE THYROID LESION	HASHIMOTOS THYROITIS	PAPILLARY HYPERPLASIA IN HASHIMOTOS THYROIDITIS	1+	FOCAL HURTHLE CELL POSITIVE <1%
27	56	F	53291	3071/16	78	11.9	2.9	SNS	MASS LESION	FOLLICULAR NEOPLASM	NODULAR GOITRE WITH PAPILLARY HYPERPLASIA	3+	80%
28	60	F	53802	2909/16	99	9.2	3.2	SNS	NODULAR LESION	COLLOID GOITRE	NODULAR ADENOMATOUS HYPERPLASIA	NEGATIVE	
29	40	F	53603	3075/16	108.3	9.2	4.6	SNS	NODULAR LESION	COLLOID GOITRE WITH CYSTIC DEGENERATION	NODULAR GOITRE WITH PAPILLARY HYPERPLASIA	NEGATIVE	
30	55	F	58747	3176/16	129.2	6	O.92	SNS	COLLOID FILLED NODULE	COLLOID NODULAR GOITRE	MULTINODULAR GOITRE WITH AREAS OF PAPILLARY HYPERPLASIA	NEGATIVE	
31		F		3461/16	96.9	3.9	8.2	MNS	MULTINODULAR GOITRE	COLLOID NODULAR GOITRE	MULTINODULAR GOITRE WITH AREAS OF PAPILLARY HYPERPLASIA	NEGATIVE	
32	45	F	84077	12_17	62.8	6.7	10.5	DTL	DIFFUSE THYROID LESION	NODULAR COLLOID GOITRE	MICROPAPILLARY CARCINOMA WITH ADENOMATOUS GOITRE	3+	75%
33	40	M		184/17	102	8.2	3.23	SNS	MASS LESION	COLLOID GOITRE	INTRACYSTIC PAPILLARY HYPERPLASIA	3+	75%
34	35	F	82191	146/17	113	7.6	4.7	SNS	NODAL METS	PAPILLARY CARCINOMA	HASHIMOTOS THYROIDITIS WITH NODAL PAPILLARY CARCINOMA METASTASIS		
35	18	F	8410	471/17	120.6	8.2	0.45	MNS	MULTINODULAR GOITRE	NODULAR GOITRE	PAPILLARY CARCINOMA	4+	90%
36	53	M	2172	475/17	113.9	7.5	4.2	SNS	PAPILLARY CARCINOMA	PAPILLARY CARCINOMA	PAPILLARY CARCINOMA	4+	90%
37	70	F	10713	765/17	132	5.2	7.5	SNS	NODULAR LESION	MULTINODULAR GOITRE	ENCAPSULATED FOLLICULAR VARIANT OF PAPILLARY CARCINOMA	NEGATIVE	

38	80	F	18482	1260/17	93.7	8.5	4.3	MNS	MULTINODULAR GOITRE	NODULAR GOITRE	COLUMNAR CEL VARIANT OF PAPILLARY CARCINOMA	4+	90%
39	47	F	22886	1306/17	87.8	7.5	3.5	SNS	COLLOID FILLED NODULE	FOLLICULAR NEOPLASM	INTRACYSTIC PAPILLARY CARCINOMA BACKGROUND OF COLLOID GOITRE	3+	70%
40	52	F	23263	1336/17	76.5	9	4.3	SNS	SOLITARY NODULAR SWELLING	FOLLICULAR NEOPLASM	FOLLICULAR VARIANT OF PAPILLARY CARCINOMA	4+	80%
41	55	F	26670	1557/17	65	6.3	0.7	SNS	MASS LESION WITH CERVICAL NODE	PAPILLARY CARCINOMA	PAPILLARY CARCINOMA WITH NODAL METASTASIS	4+	90%
42	48	F	41373	2241/16	89.9	4.5	7.3	DTL	DIFFUSE THYROID LESION	PAPILLARY CARCINOMA	PAPILLARY CARCINOMA THYROID	4+	90%
43	27	F	27391	1583/17	67.8	6.9	0.9	MNS	NODULAR GOITRE	NODULAR COLLOID GOITRE	ENCAPSULATED FOLLICULAR VARIANT OF PAPILLARY CARCINOMA	3+	75%
44	45	F	14566	990/17	133	14.6	0.9	DTL	DIFFUSE THYROID LESION	HYPERPLASTIC GOITRE	HASHIMOTOS THYROIDITIS WITH TOXIC CHANGE	NEGATIVE	
45	31	F	124576	1361/17	112	7.9	1.8	DTL	HYPERTHYROID STATE		GRAVES DISEASE	NEGATIVE	
46	33	F	269421	1469/17	67.9	5.6	4.9	SNS	SOLITARY NODULAR SWELLING	COLLOID GOITRE	C0LLOID GOITRE WITH CYSTIC DEGENERATION	NEGATIVE	